

WEST Search History

Search Paper 14

DATE: Wednesday, July 24, 2002

<u>Set Name</u> side by side	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u> result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
L7	L6 and zinc	1	L7
L6	joung-j\$	58	L6
L5	l4	5	L5
L4	L3 not l2	5	L4
L3	pabo-carl\$.in.	17	L3
L2	pabo-carl.in.	12	L2
L1	joung-j.in.	11	L1

END OF SEARCH HISTORY

WEST Search History

DATE: Wednesday, July 24, 2002

<u>Set Name</u> side by side	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u> result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
L2	L1 same (cell or vivo)	29	L2
L1	(zinc finger) same random\$	111	L1

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Search Results - Record(s) 1 through 29 of 29 returned.

☐ 1. Document ID: US 20020061512 A1

L2: Entry 1 of 29

File: PGPB

May 23, 2002

PGPUB-DOCUMENT-NUMBER: 20020061512

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020061512 A1

TITLE: Zinc finger domains and methods of identifying same

PUBLICATION-DATE: May 23, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kim, Jin-Soo	Taejon		KR	
Kwon, Yong Do	Incheon		KR	
Kim, Hyun-Won	Seoul		KR	
Ryu, Eun-Hyun	Taejon		KR	
Hwang, Moon-Sun	Taejon		KR	

US-CL-CURRENT: 435/4; 435/226, 530/326, 536/23.5

ABSTRACT:

Disclosed is an in vivo selection method for identifying zinc finger domains that recognize any given target site. Also disclosed are the amino acid sequences of zinc finger domains that recognize particular sites.

L2: Entry 1 of 29

File: PGPB

May 23, 2002

DOCUMENT-IDENTIFIER: US 20020061512 A1

TITLE: Zinc finger domains and methods of identifying same

Detail Description Paragraph (117):

[0154] In one instance, yeast cells were first transformed with a reporter plasmid containing the composite binding sequence 5'-GAGCGGGCG-3' (the 4-bp target sequence is underlined), which was operably linked to the reporter gene. Then, the plasmid library of mutant zinc finger domains prepared by random mutagenesis was introduced into the transformed yeast cells. About 10.^{sup.6} colonies were obtained in medium lacking both leucine and tryptophan. Because the reporter plasmid and the zinc finger domain expression plasmids contain yeast LEU2 and TRP1 genes, respectively, as a marker, yeast cells were grown in medium lacking both leucine and tryptophan in order to select for cells that contain both the reporter and the zinc finger domain expression plasmid.

Detail Description Paragraph (118):

[0155] In one implementation, the library of zinc finger domains derived from the human genome was transformed into cells bearing the reporter plasmids. The transformation was performed on five different host cell strains, each strain containing one of five different target sequences operably linked to the reporter gene. About 10.^{sup.5} colonies were obtained per transformation in medium lacking

both leucine and tryptophan. Transformants were grown on petri plates containing synthetic medium lacking leucine and tryptophan. After incubation, transformed cells were collected by applying a 10% sterile glycerol solution to the plates, scraping the colonies into the solution, and retrieving the solution. Cells were stored as frozen aliquots in the glycerol solution. A single aliquot was spread onto medium lacking leucine, tryptophan and histidine. 3-aminotriazole (AT) was added to the growth medium at the final concentrations of 0, 0.03, 0.1 and 0.3 mM. AT is a competitive inhibitor of His3 and titrates the sensitivity of the HIS3 selection system. AT suppressed the basal activity of His3. Such basal activity can arise from leaky expression of the HIS3 gene on the reporter plasmid. Out of about 10^{sup.7} yeast cells spread on medium, on the order of hundreds of colonies grew in the selective medium lacking AT. The number of colonies gradually decreased as the concentration of AT increased. On the order of tens of colonies grew in the selective medium containing 0.3 mM of AT. Several colonies were randomly picked from the medium lacking AT and from the medium containing 0.3 mM of AT. Plasmids were isolated from yeast cells and transformed into Escherichia coli strain KC8 (pyrF leuB600 trpC hisB463). The plasmids encoding zinc finger transcription factor were isolated, and the DNA sequences of selected zinc finger domains were determined.

Detail Description Paragraph (142):

[0176] The KTNr zinc finger domain isolated from the random mutant library was originally selected with the GAGC reporter plasmid. As expected, colonies were formed only with the GAGC plasmid. Zinc finger domains obtained from the library derived from the human genome also showed expected specificity. For example, HSNK, which had been selected with the GACT plasmid, allowed cell growth only with the GACT plasmid when retransformed into yeast cells. VSTR, which had been selected with the GCTT plasmid, showed the highest activity with the GCTT plasmid. RDER, which was selected with the GAGT plasmid, has the same amino acid residues at the four base-contacting positions as does finger 3 of Zif268. As expected, this zinc finger domain showed sequence specificity similar to that of finger 3. SSNR, selected with the GAGC and GAGT plasmids, allowed cell growth on histidine-deficient medium with the GAGC plasmid but not with the GAGT plasmid. QSTV, obtained with the ACAT plasmid, did not allow cell growth with any of the plasmids tested in this assay. However, this zinc finger domain was able to bind to the ACAT sequence tightly in vitro as demonstrated below.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: US 20020056144 A1

L2: Entry 2 of 29

File: PGPB

May 9, 2002

PGPUB-DOCUMENT-NUMBER: 20020056144

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020056144 A1

TITLE: Transgenic animal models for cardiac hypertrophy and methods of use thereof (screening)

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Grant, Stephen R.	Ft. Worth	TX	US	
Olson, Eric N.	Dallas	TX	US	

US-CL-CURRENT: 800/3; 435/4

ABSTRACT:

Transgene constructs for generating transgenic animals, wherein the transgene encodes a gene product which modulates transcription of a hypertrophy-sensitive gene, are provided. Further provided are recombinant vectors comprising the transgenes of the invention. Further provided are transgenic animals generated using the transgene constructs. Further provided are enzyme-based, cell-based, and whole-animal-based assays for detecting substances having therapeutic activity toward cardiac hypertrophy. Further provided are compositions comprising substances which modulate levels of active product of a hypertrophy-sensitive gene. Further provided are methods of treating cardiac hypertrophy.

L2: Entry 2 of 29

File: PGPB

May 9, 2002

DOCUMENT-IDENTIFIER: US 20020056144 A1

TITLE: Transgenic animal models for cardiac hypertrophy and methods of use thereof (screening)

Detail Description Paragraph (129):

[0179] As an example of protein interactive trapping, a yeast two-hybrid screen can be performed using mouse GATA4 as bait to identify potential interacting factors in the adult myocardium, as described. Molkenstein et al. (1998) Cell. 93:215-228. In this example, the GATA4 bait contains amino acids 130-409 fused in-frame with the GAL4 DNA binding domain. This region of GATA4 encompasses the two zinc finger domains and is encoded within a PstI-NsiI fragment, which was cloned into a Pst I site in the pAS yeast expression vector. pAS-GATA4 was co-transformed into yeast with an adult mouse heart library that contained the GAL4 activation domain fused to random cDNAs and over 5 million primary colonies were screened for positive interactions. Approximately 100 positive yeast colonies were initially identified. From each individual colony, the activating plasmid was rescued and the cDNA insert was sequenced. Clones containing cDNA inserts in the antisense orientation or out-of-frame were discarded. The remaining clones (approximately 21) were retransformed back into yeast to test for specificity.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	RWC	Draw Desc	Image
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☐ 3. Document ID: US 20020048792 A1

L2: Entry 3 of 29

File: PGPB

Apr 25, 2002

PGPUB-DOCUMENT-NUMBER: 20020048792

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020048792 A1

TITLE: Methods and materials for regulated production of proteins

PUBLICATION-DATE: April 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Natesan, Sridaran	Chestnut Hill	MA	US	
Clackson, Timothy P.	Cambridge	MA	US	
Pollock, Roy M.	Medford	MA	US	

US-CL-CURRENT: 435/69.1; 435/320.1, 435/455

ABSTRACT:

This invention provides methods and materials for regulated production of proteins.

L2: Entry 3 of 29

File: PGPB

Apr 25, 2002

DOCUMENT-IDENTIFIER: US 20020048792 A1

TITLE: Methods and materials for regulated production of proteins

Detail Description Paragraph (85):

[0114] For additional examples, information and guidance on designing, mutating, selecting, combining and characterizing DNA binding domains, see, e.g., Pomerantz J L, Wolfe S A, Pabo C O, Structure-based design of a dimeric zinc finger protein Biochemistry 1998 Jan 27;37(4):965-970; Kim J-S and Pabo C O, Getting a Handhold on DNA: Design of Poly-Zinc Finger Proteins with Femtomolar Dissociation Constants, PNAS USA, 1998 Mar 17;95(6):2812-2817; Kim J S, Pabo C O, Transcriptional repression by zinc finger peptides. Exploring the potential for applications in gene therapy. , J Biol Chem 1997 Nov 21;272(47):29795-29800; Greisman H A, Pabo C O , A general strategy for selecting high-affinity zinc finger proteins for diverse DNA target sites, Science 1997 Jan 31;275(5300):657-661; Rebar E.J, Greisman H.A, Pabo C.O, Phage display methods for selecting zinc finger proteins with novel DNA-binding specificities, Methods Enzymol 1996;267:129-149; Pomerantz J.L, Pabo C.O, Sharp P.A, Analysis of homeodomain function by structure-based design of a transcription factor, Proc Natl Acad Sci USA 1995 Oct 10;92(21):9752-9756; Rebar E J, Pabo C O, Zinc finger phage: affinity selection of fingers with new DNA-binding specificities, Science 1994, Feb 4;263:671-673; Choo Y, Sanches-Garcia I, Klug A, In vivo repression by a site-specific DNA-binding protein designed against an oncogenic sequence, Nature 1994, Dec 15;372:642-645; Choo Y, Klug A, Toward a code for the interaction of zinc fingers with DNA: Selection of randomized fingers displayed on phage, PNAS USA, Nov 1994; 91:11163-11167; Wu H, Yang W-P, Barbas C F III, Building zinc fingers by selection: toward a therapeutic application, PNAS USA January 1995; 92:344-348; Jamieson A C, Kim S-H, Wells J A, In Vitro selection of zinc fingers with altered DNA-binding specificity, Biochemistry 1994, 33:5689-5695; International patent applications WO 96/20951, WO 94/18317, WO 96/06166 and WO 95/19431; and U.S. Ser. No. 60/084819.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 4. Document ID: US 20020045158 A1

L2: Entry 4 of 29

File: PGPB

Apr 18, 2002

PGPUB-DOCUMENT-NUMBER: 20020045158

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045158 A1

TITLE: Cells for drug discovery

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Case, Casey	San Mateo	CA	US	

US-CL-CURRENT: 435/4; 435/325

ABSTRACT:

Disclosed herein are compositions and method useful in screening a compound for its interaction and/or effect with a molecular target and/or cellular process.

L2: Entry 4 of 29

File: PGPB

Apr 18, 2002

DOCUMENT-IDENTIFIER: US 20020045158 A1

TITLE: Cells for drug discovery

Detail Description Paragraph (92):

[0115] Test cells are preferably generated by regulation of cellular genes with zinc finger proteins or fusion molecules described herein. In some methods, a test cell is produced by engineering a cell to express an exogenous zinc finger protein (or fusion molecule) designed to repress expression of an endogenous molecular target. In alternative methods, a test cell is produced by engineering a cell to express an exogenous zinc finger protein (or fusion molecule) designed to activate or increase expression of an endogenous molecular target. The resulting test cells are preferably substantially identical to the corresponding control cells except for an exogenous nucleic acid encoding the exogenous zinc finger-containing molecule (and, possibly, a low incidence of random mutations resulting from environmental factors). Thus, in certain embodiments, the phenotype of the test and controls cell populations will differ only in regard to the levels of the protein(s) subject to regulation by the exogenous zinc finger-containing molecule (and other secondary changes resulting from regulation of that protein). In other embodiments, the test and control cells may not be substantially identical but, in these cases, the genetic differences (besides any exogenous ZFP-coding polynucleotides) are typically known.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 5. Document ID: US 20020042138 A1

L2: Entry 5 of 29

File: PGPB

Apr 11, 2002

PGPUB-DOCUMENT-NUMBER: 20020042138

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020042138 A1

TITLE: Delta-erythroid kruppel-like factors and methods of use

PUBLICATION-DATE: April 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Townes, Tim M.	Birmingham	AL	US	
Donze, David	Silver Springs	MD	US	

US-CL-CURRENT: 435/456; 424/93.21, 435/372

ABSTRACT:

The invention features .delta.-erythroid kruppel-like factors (.delta.-EKLFs), and methods of using nucleic acids encoding .delta.-EKLFs to increase .delta.-globin gene expression in a cell.

L2: Entry 5 of 29

File: PGPB

Apr 11, 2002

DOCUMENT-IDENTIFIER: US 20020042138 A1

TITLE: Delta-erythroid kruppel-like factors and methods of use

Detail Description Paragraph (10):

[0034] Amino acid positions -1 and 2-6 are randomized in each of the three

.beta.-EKLF zinc fingers (see FIG. 2; also see Miller et al., Mol. Cell. Biol. 13(5):2776 for the nucleotide and amino acid sequences of .beta.-EKLF), and variants that specifically bind nucleic acids containing .delta.-globin promoter sequences (e.g., 5'-TGA AAC CCT-3' or 5'-CTA ATG AAA-3') are selected. Because of limitations of the phage display method, preferably, one zinc finger is randomized and selected at a time. For example, beginning with the first .beta.-EKLF zinc finger (see FIG. 2), overlapping oligonucleotides spanning the coding region of the zinc finger, and containing all four bases at the positions corresponding to amino acids -1 and 2-6, are annealed and extended with DNA polymerase (e.g., Sequenase 2.0, United States Biochemical) to create a library of DNA fragments that encode a mixture of all possible amino acid combinations at amino acid positions -1 and 2-6. These fragments are linked to the second and third zinc fingers of .beta.-EKLF, and cloned into the vector fUSE5 (Smith et al., Meth. Enz. 217:228, 1993). The resulting random library is then transformed into an appropriate E. coli strain, such as MC1061 (Smith et al., Methods Enzymol. 217:228, 1993), and a collection of bacteriophage are produced, which express a modified .beta.-EKLF in their coat protein. In these modified .beta.-EKLFs, the first zinc finger contains random sequences in amino acid positions -1 and 2-6, and the second and third zinc fingers are wild type.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 6. Document ID: US 20010049104 A1

L2: Entry 6 of 29

File: PGPB

Dec 6, 2001

PGPUB-DOCUMENT-NUMBER: 20010049104

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010049104 A1

TITLE: Methods for modulating cellular and organismal phenotypes

PUBLICATION-DATE: December 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Stemmer, Willem P.C.	Los Gatos	CA	US	
Minshull, Jeremy	Menlo Park	CA	US	
Keenan, Robert J.	San Francisco	CA	US	

US-CL-CURRENT: 435/6; 435/455

ABSTRACT:

Methods for identifying and controlling the genetic and metabolic pathways underlying complex phenotypes are provided. Conjoint polynucleotide segments that contribute to or disrupt elements of a multigenic phenotype are produced and expressed in cells of interest. Conjoint polynucleotide segments are recombined and/or mutated to give rise to libraries of recombinant concatamers which are expressed in cells of interest. Libraries of conjoint polynucleotide segments and recombinant concatamers are expressed episomally or integrated into the DNA of organelles or chromosomes. Cells are screened or selected to identify members of the population of cells exhibiting a desired phenotype. Libraries and vectors comprising conjoint polynucleotide segments and recombinant concatamers, as well as cells expressing such libraries and vectors or their components are provided. Kits containing conjoint polynucleotide segments, recombinant concatamers, vectors including such polynucleotides, and cells including such polynucleotides and vectors are provided.

L2: Entry 6 of 29

File: PGPB

Dec 6, 2001

DOCUMENT-IDENTIFIER: US 20010049104 A1

TITLE: Methods for modulating cellular and organismal phenotypes

Detail Description Paragraph (58):

[0086] Typically, to facilitate manipulation, the multiple segments are placed under regulatory control of a single promoter and/or enhancer selected to control expression in the cell type (or organism) of interest. Alternatively, each segment can be placed under independent regulatory control. The short polynucleotide sequences can be DNA or RNA, and expressed in either the sense (coding) or the antisense ("anticoding") direction. Alternatively, the polynucleotide segments can be e.g., cDNAs, minigenes, genomic DNA segments, or synthetic DNA sequences such as randomly selected aptamers, random or partially random N-mers, or synthesized consensus sequences. In other embodiments, DNA molecules encoding RNA molecules including ribozymes, tRNAs, components of RNPs, and components of the enzymatic splicing machinery can be used. Alternatively, DNA molecules encoding structural proteins, or domains or subsequences thereof, of such cellular targets as transcription factors, e.g., zinc finger proteins, enzymes, receptors, polypeptide hormones, and the like are employed. In some instances, sequences that are not expressed in a mature protein, e.g., introns, inteins, are included among the elements of conjoint polynucleotide segments.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 7. Document ID: US 6410271 B1

L2: Entry 7 of 29

File: USPT

US-PAT-NO: 6410271

DOCUMENT-IDENTIFIER: US 6410271 B1

TITLE: Generation of highly diverse library of expression vectors via homologous recombination in yeast

DATE-ISSUED: June 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zhu; Li	Palo Alto	CA		
Hua; Shaobing Benjamin	Cupertino	CA		

US-CL-CURRENT: 435/69.7; 435/483, 435/69.1, 435/7.1, 435/71.1

ABSTRACT:

Methods are provided for generating highly diverse libraries of expression vectors encoding fusion proteins such as single-chain antibodies via homologous recombination in yeast. The method comprises: transforming into yeast cells a linearized yeast expression vector having a 5'- and 3'-terminus sequence at the site of linearization and a library of insert nucleotide sequences that are linear and double-stranded; and having homologous recombination occur between the vector and the insert sequence such that the insert sequence is included in the vector in the transformed yeast cells. The insert sequence comprises a first nucleotide sequence encoding a first polypeptide subunit, a second nucleotide sequence encoding a second polypeptide subunit, a linker sequence encoding a linker peptide that links the first and second polypeptide subunits, and a 5'- and 3'-flanking sequence at the ends of the insert sequence which are sufficiently homologous to the 5'- and 3'-terminus sequences of the linearized yeast expression vector, respectively, to enable homologous recombination to occur. The first polypeptide subunit, the second

polypeptide subunit, and the linker polypeptide are expressed as a single fusion protein; and the first and second nucleotide sequences each independently varies within the library of expression vectors.

25 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

L2: Entry 7 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6410271 B1

TITLE: Generation of highly diverse library of expression vectors via homologous recombination in yeast

Detailed Description Text (218):

Optionally, a collection of certain domain structures, such as zinc finger and helix-loop-helix protein domains, may be inserted into the AD-containing expression vector in yeast cell via homologous recombination. The yeast clones containing the vector with BD fused to each domain structure may be arrayed in multiple-well plates and screened against the scFv library for affinity binding between the scFv and each domain structure. The domain structure may be 18-20 amino acids at length and its sequence may not be totally random. Such a collection of domain structures may be generated by using synthetic oligonucleotides with characteristic conserved and random/degenerate residues to cover most of the rational domain structures.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 8. Document ID: US 6410246 B1

L2: Entry 8 of 29

File: USPT

US-PAT-NO: 6410246

DOCUMENT-IDENTIFIER: US 6410246 B1

TITLE: Highly diverse library of yeast expression vectors

DATE-ISSUED: June 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zhu; Li	Palo Alto	CA		
Hua; Shaobing Benjamin	Cupertino	CA		

US-CL-CURRENT: 435/7.1; 435/320.1, 435/7.31, 536/23.4, 536/23.53

ABSTRACT:

A highly diverse library of yeast expression vectors encoding a library of fusion proteins such as antibodies is provided. The yeast expression vector formed in the library comprises: a first nucleotide sequence encoding a first polypeptide subunit; a second nucleotide sequence encoding a second polypeptide subunit; and a linker sequence encoding a linker peptide that links the first nucleotide sequence and the second nucleotide sequence. The first polypeptide subunit, the second polypeptide subunit, and the linker polypeptide are expressed as a single fusion protein within the library of fusion proteins. The first and second nucleotide sequences each independently varies within the library of expression vectors. The library of fusion proteins expressed by the library expression vectors can be used for screening against target molecules such as proteins, peptides, DNAs and small molecules in

vitro and in vivo.

- 32 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

L2: Entry 8 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6410246 B1

TITLE: Highly diverse library of yeast expression vectors

Detailed Description Text (218):

Optionally, a collection of certain domain structures, such as zinc finger and helix-loop-helix protein domains, may be inserted into the AD-containing expression vector in yeast cell via homologous recombination. The yeast clones containing the vector with BD fused to each domain structure may be arrayed in multiple-well plates and screened against the scFv library for affinity binding between the scFv and each domain structure. The domain structure may be 18-20 amino acids at length and its sequence may not be totally random. Such a collection of domain structures may be generated by using synthetic oligonucleotides with characteristic conserved and random/degenerate residues to cover most of the rational domain structures.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 9. Document ID: US 6406863 B1

L2: Entry 9 of 29

File: USPT

US-PAT-NO: 6406863

DOCUMENT-IDENTIFIER: US 6406863 B1

TITLE: High throughput generation and screening of fully human antibody repertoire in yeast

DATE-ISSUED: June 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zhu; Li	Palo Alto	CA		
Hua; Shaobing Benjamin	Cupertino	CA		

US-CL-CURRENT: 435/7.1; 435/29, 435/69.7, 435/7.8, 435/71.1

ABSTRACT:

Compositions, kits and methods are provided for generating highly diverse libraries of proteins such as antibodies via homologous recombination in vivo, and screening these libraries against protein, peptide and nucleic acid targets using a two-hybrid method in yeast. The method for screening a library of tester proteins against a target protein or peptide comprises: expressing a library of tester proteins in yeast cells, each tester protein being a fusion protein comprised of a first polypeptide subunit whose sequence varies within the library, a second polypeptide subunit whose sequence varies within the library independently of the first polypeptide, and a linker peptide which links the first and second polypeptide subunits; expressing one or more target fusion proteins in the yeast cells expressing the tester proteins, each of the target fusion proteins comprising a target peptide or protein; and selecting those yeast cells in which a reporter gene is expressed, the expression of the reporter gene being activated by binding of the

tester fusion protein to the target fusion protein.

- 26 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

L2: Entry 9 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6406863 B1

TITLE: High throughput generation and screening of fully human antibody repertoire in yeast

Detailed Description Text (219):

Optionally, a collection of certain domain structures, such as zinc finger and helix-loop-helix protein domains, may be inserted into the AD-containing expression vector in yeast cell via homologous recombination. The yeast clones containing the vector with BD fused to each domain structure may be arrayed in multiple-well plates and screened against the scFv library for affinity binding between the scFv and each domain structure. The domain structure may be 18-20 amino acids at length and its sequence may not be totally random. Such a collection of domain structures may be generated by using synthetic oligonucleotides with characteristic conserved and random/degenerate residues to cover most of the rational domain structures.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 10. Document ID: US 6365344 B1

L2: Entry 10 of 29

File: USPT

US-PAT-NO: 6365344

DOCUMENT-IDENTIFIER: US 6365344 B1

TITLE: Methods for screening for transdominant effector peptides and RNA molecules

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nolan; Garry P.	Menlo Park	CA		
Rothenberg; S. Michael	Palo Alto	CA		

US-CL-CURRENT: 435/6; 435/320.1, 435/69.1, 536/23.4

ABSTRACT:

Biochemical libraries are screened for transdominant intracellularly bioactive agents by expressing a molecular library of randomized nucleic acids as a plurality of corresponding expression products in a plurality of cells, each of the nucleic acids comprising a different nucleotide sequence, detecting a cell of the plurality of cells exhibiting a changed physiology in response to the presence in the cell of a transdominant expression product of the corresponding expressio products; and isolating the cell and/or transdominant expression product.

12 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

L2: Entry 10 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6365344 B1

TITLE: Methods for screening for transdominant effector peptides and RNA molecules

Detailed Description Text (5):

It may also be desirable to provide a partner which conformationally restricts the randomized expression product to more specifically define the number of structural conformations available to the cell. For example, such a partner may be a synthetic presentation structure: an artificial polypeptide capable of intracellularly presenting a randomized peptide as a conformation-restricted domain. Generally such presentation structures comprise a first portion joined to the N-terminal end of the randomized peptide, and a second portion joined to the C-terminal end of the peptide. Preferred presentation structures maximize accessibility to the peptide by presenting it on an exterior loop, for example of coiled-coils, (Myszka, D. G., and Chaiken, I. M. Design and characterization of an intramolecular antiparallel coiled coil peptide. Biochemistry. 1994. 33:2362-2372). To increase the functional isolation of the randomized expression product, the presentation structures are selected or designed to have minimal biologically active as expressed in the target cell. In addition, the presentation structures may be modified, randomized, and/or matured to alter the presentation orientation of the randomized expression product. For example, determinants at the base of the loop may be modified to slightly modify the internal loop peptide tertiary structure, while maintaining the absolute amino acid identity. Other presentation structures include zinc-finger domains, loops on beta-sheet turns and coiled-coil stem structures in which non-critical residues are randomized; loop structures held together by cysteine bridges, cyclic peptides, etc.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 11. Document ID: US 6350932 B1

L2: Entry 11 of 29

File: USPT

US-PAT-NO: 6350932

DOCUMENT-IDENTIFIER: US 6350932 B1

TITLE: Vitamin D receptor ablated mice

DATE-ISSUED: February 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Demay; Marie	Wellesley	MA		
Li; Yan Chun	Chicago	IL		

US-CL-CURRENT: 800/3, 435/325, 435/354, 435/455, 435/463, 514/880, 800/13, 800/14, 800/18, 800/8, 800/9

ABSTRACT:

A transgenic mouse containing disruptions in both vitamin D alleles and lacking vitamin D receptor activity is described. The transgenic mouse displays perioral and periorbital alopecia, hypocalcemia, hypophosphatemia, and bone demineralization. The transgenic mouse is useful for screening treatments for a number of conditions associated with vitamin D receptor related disorders including skin disorders, immune system disorders, and proliferative disorders.

10 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

L2: Entry 11 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6350932 B1

TITLE: Vitamin D receptor ablated mice

Brief Summary Text (62):

As used herein, "disruption of a gene" refers to a change in the gene sequence, e.g., a change in the coding region. Disruption includes: insertions, deletions, point mutations, and rearrangements, e.g., inversions. The disruption can occur in a region of the native vitamin D receptor DNA sequence (e.g., one or more exons) and/or the promoter region of the gene so as to decrease or prevent expression of the gene in a cell as compared to the wild-type or naturally occurring sequence of the gene. The "disruption" can be induced by classical random mutation or by site directed methods. Disruptions can be transgenically introduced. The deletion of an entire gene is a disruption. The disruption can, e.g., occur in a region of the VDR which mediates heterodimerization, in a region which mediated DNA binding, e.g., a zinc-finger domain, or in a region which mediates ligand binding. Preferred disruptions reduce VDR levels to about 50% of wild type, in heterozygotes or essentially eliminate VDR in homozygotes.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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KWIC	Draw Desc	Image
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☐ 12. Document ID: US 6306650 B1

L2: Entry 12 of 29

File: USPT

US-PAT-NO: 6306650

DOCUMENT-IDENTIFIER: US 6306650 B1

TITLE: Nucleic acid molecule encoding a .beta.-erythroid kruppel-like factor that binds to a .delta.-globin promoter

DATE-ISSUED: October 23, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Townes; Tim M.	Birmingham	AL		
Donze; David	Silver Spring	MD		

US-CL-CURRENT: 435/325; 435/243, 435/320.1, 435/410, 536/23.5

ABSTRACT:

The invention features .delta.-erythroid kruppel-like factors (.delta.-EKLFs), and methods of using nucleic acids encoding .delta.-EKLFs to increase .delta.-globin gene expression in a cell.

10 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

L2: Entry 12 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6306650 B1

TITLE: Nucleic acid molecule encoding a .beta.-erythroid kruppel-like factor that binds to a .delta.-globin promoter

- Detailed Description Text (10):

Amino acid positions -1 and 2-6 are randomized in each of the three .beta.-EKLF zinc fingers (see FIG. 2; also see Miller et al., Mol. Cell. Biol. 13(5):2776 for the nucleotide and amino acid sequences of .beta.-EKLF), and variants that specifically bind nucleic acids containing .delta.-globin promoter sequences (e.g., 5'-TGA AAC CCT-3' or 5'-CTA ATG AAA-3') are selected. Because of limitations of the phage display method, preferably, one zinc finger is randomized and selected at a time. For example, beginning with the first .beta.-EKLF zinc finger (see FIG. 2), overlapping oligonucleotides spanning the coding region of the zinc finger, and containing all four bases at the positions corresponding to amino acids -1 and 2-6, are annealed and extended with DNA polymerase (e.g., Sequenase 2.0, United States Biochemical) to create a library of DNA fragments that encode a mixture of all possible amino acid combinations at amino acid positions -1 and 2-6. These fragments are linked to the second and third zinc fingers of .beta.-EKLF, and cloned into the vector fUSE5 (Smith et al., Meth. Enz. 217:228, 1993). The resulting random library is then transformed into an appropriate E. Coli strain, such as MC1061 (Smith et al., Methods Enzymol. 217:228, 1993), and a collection of bacteriophage are produced, which express a modified .beta.-EKLF in their coat protein. In these modified .beta.-EKLFs, the first zinc finger contains random sequences in amino acid positions -1 and 2-6, and the second and third zinc fingers are wild type.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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RMAC	Draw Desc	Image
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☐ 13. Document ID: US 6242568 B1

L2: Entry 13 of 29

File: USPT

US-PAT-NO: 6242568

DOCUMENT-IDENTIFIER: US 6242568 B1

TITLE: Zinc finger protein derivatives and methods therefor

DATE-ISSUED: June 5, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barbas, III; Carlos F.	San Diego	CA		
Gottesfeld; Joel M.	San Diego	CA		
Wright; Peter E.	La Jolla	CA		

US-CL-CURRENT: 530/350; 435/252.3, 435/320.1, 435/417, 435/69.1, 530/400, 536/23.5, 536/23.6, 536/23.72

ABSTRACT:

The present invention provides zinc finger nucleotide binding polypeptide variants that have at least two zinc finger modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide sequence. Also provided are methods of use of such zinc finger nucleotide binding polypeptide variants and methods for isolating the same using expression libraries encoding the polypeptide variants containing randomized substitutions of amino acids. Exemplary zinc finger nucleotide binding polypeptide variants of the invention include two cysteines and two histidines whereby both cysteines are amino proximal to both histidines.

56 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

L2: Entry 13 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6242568 B1

TITLE: Zinc finger protein derivatives and methods therefor

Brief Summary Text (19):

In yet a further embodiment, the invention provides a method for obtaining an isolated zinc finger-nucleotide binding polypeptide variant which binds to a cellular nucleotide sequence comprising identifying the amino acids in a zinc finger-nucleotide binding polypeptide that bind to a first cellular nucleotide sequence and modulate the function of the nucleotide sequence; creating an expression library encoding the polypeptide variant containing randomized substitution of the amino acids identified; expressing the library in a suitable host cell; and isolating a clone that produces a polypeptide variant that binds to a second cellular nucleotide sequence and modulates the function of the second nucleotide sequence. Preferably, the expression library encoding the polypeptide variant is a phage display library.

Detailed Description Text (102):

In general, surface membrane proteins which bind to specific effector molecules are referred to as receptors. In the present invention, antibodies are preferred receptors. Antibodies can be used to target liposomes to specific cell-surface ligands. For example, certain antigens expressed specifically on tumor cells, referred to as tumor-associated antigens (TAAs), may be exploited for the purpose of targeting antibody-zinc finger-nucleotide binding protein-containing liposomes directly to the malignant tumor. Since the zinc finger-nucleotide binding protein gene product may be indiscriminate with respect to cell type in its action, a targeted delivery system offers a significant improvement over randomly injecting non-specific liposomes. A number of procedures can be used to covalently attach either polyclonal or monoclonal antibodies to a liposome bilayer. Antibody-targeted liposomes can include monoclonal or polyclonal antibodies or fragments thereof such as Fab, or F(ab')₂, as long as they bind efficiently to an the antigenic epitope on the target cells. Liposomes may also be targeted to cells expressing receptors for hormones or other serum factors.

Detailed Description Text (103):

In another embodiment, the invention provides a method for obtaining an isolated zinc finger-nucleotide binding polypeptide variant which binds to a cellular nucleotide sequence comprising, first, identifying the amino acids in a zinc finger-nucleotide binding polypeptide that bind to a first cellular nucleotide sequence and modulate the function of the nucleotide sequence. Second, an expression library encoding the polypeptide variant containing randomized substitution of the amino acids identified in the first step is created. Third, the library is expressed in a suitable host cell, which will be apparent to those of skill in the art, and finally, a clone is isolated that produces a polypeptide variant that binds to a second cellular nucleotide sequence and modulates the function of the second nucleotide sequence. The invention also includes a zinc finger-nucleotide binding polypeptide variant produced by the method described above.

Detailed Description Text (169):

Phagemid libraries for expressing each of the randomized zinc fingers of this invention were prepared in the following procedure. To form circularized vectors containing the PCR product insert, 640 ng of the digested PCR products were admixed with 2 ug of the linearized pComb3.5 phagemid vector and ligation was allowed to proceed overnight at room temperature using 10 units of BRL ligase (Gaithersburg, Md.) in BRL ligase buffer in a reaction volume of 150 ul. Five separate ligation reactions were performed to increase the size of the phage library having randomized zinc fingers. Following the ligation reactions, the circularized DNA was precipitated at -20.degree. C. for 2 hours by the admixture of 2 ul of 20 mg/ml glycogen, 15 ul of 3 M sodium acetate at pH 5.2 and 300 ul of ethanol. DNA was then pelleted by microcentrifugation at 4.degree. C. for 15 minutes. The DNA pellet was washed with cold 70% ethanol and dried under vacuum. The pellet was resuspended in 10 ul of water and transformed by electroporation into 300 ul of E. coli XL1-Blue

cells to form a phage library.

Detailed Description Text (173):

For determining the titering colony forming units (cfu), phage (packaged phagemid) were diluted in SB and 1 ul was used to infect 50 ul of fresh (A.sub.OD600 =1) E. coli XL 1-Blue cells grown in SB containing 10 ug/ml tetracycline. Phage and cells were maintained at room temperature for 15 minutes and then directly plated on LB/carbenicillin plates. The randomized zinc finger 3 library consisted of 5.times.10.sup.7 PFU total.

Detailed Description Text (179):

Following each round of panning, the percentage yield of phage were determined, where % yield=(number of phage eluted/number of phage applied).times.100. The initial phage input ratio was determined by titering on selective plates to be approximately 10.sup.11 cfu for each round of panning. The final phage output ratio was determined by infecting two ml of logarithmic phase XL1-Blue cells as described above and plating aliquots on selective plates. From this procedure, clones were selected from the Fab library for their ability to bind to the new binding sequence oligo. The selected clones had randomized zinc finger 3 domains.

Detailed Description Text (190):

Genes encoding wild-type zinc-finger proteins were placed under the control of the Salmonella typhimurium araB promoter by insertion of a DNA fragment amplified by the polymerase chain reaction (PCR) and containing the wild-type Zif268 gene of pzif89 (Pavletich, supra) with the addition of multiple restriction sites (XhoI/SacI and XbaI/SpeI). The resulting plasmid vector was subsequently used for subcloning the selected zinc-finger genes for immunoscreening. In this vector the zinc finger protein is expressed as a fusion with a hemagglutinin decapeptide tag at its C-terminus which may be detected with an anti-decapeptide monoclonal antibody (FIG. 8A) (Field, et al., Mol. & Cell. Biol. 8:2159-2165, 1988). The Zif268 protein is aligned to show the conserved features of each zinc finger. The .alpha.-helices and antiparallel .beta.-sheets are indicated. Six amino-acid residues underlined in each finger sequence were randomized in library constructions. The C-terminal end of Zif268 protein was fused with a fragment containing a decapeptide tag. The position of fusion is indicated by an arrow.

Detailed Description Text (214):

Following selection for binding the native consensus or HIV-1 target sequences, functional zinc fingers were rapidly identified with an immunoscreening assay. Expression of the selected proteins in a pAraHA derivative resulted in the fusion of the mutant Zif268 proteins with a peptide tag sequence recognized by a monoclonal antibody (FIG. 8A). Binding was determined in an ELISA format using crude cell lysates. A qualitative assessment of specificity can also be achieved with this methodology which is sensitive to at least 4-fold differences in affinity. Several positive clones from each selection were sequenced and are shown in FIG. 9. The six randomized residues of finger 1 and 3 are at positions -1, 2, 3, 4, 5, and 6 in the .alpha.-helical region, and at -2, -1, 1, 2, 3, and 4 in finger 2 (FIG. 9). The three nucleotides denote the binding site used for affinity selection of each finger. Proteins studied in detail are indicated with a clone designation.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 14. Document ID: US 6225451 B1

L2: Entry 14 of 29

File: USPT

US-PAT-NO: 6225451

DOCUMENT-IDENTIFIER: US 6225451 B1

TITLE: Chromosome 11-linked coronary heart disease susceptibility gene CHD1

DATE-ISSUED: May 1, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ballinger; Dennis G.	Menlo Park	CA		
Ding; Wei	Salt Lake City	UT		
Wagner; Susanne	Murray	UT		
Hess; Mark A.	Salt Lake City	UT		

US-CL-CURRENT: 536/22.1; 435/6

ABSTRACT:

Human coronary heart disease susceptibility gene (CHD1), some alleles of which are related to susceptibility to coronary heart disease. Germline mutations in the CHD1 gene and their use in the diagnosis of predisposition to coronary heart disease and to metabolic disorders, including hypoalphalipoproteinemia, familial combined hyperlipidemia, insulin resistant syndrome X or multiple metabolic disorder, obesity, diabetes and dyslipidemic hypertension. Presymptomatic therapy of individuals who carry deleterious alleles of the CHD1 gene (including gene therapy, protein replacement therapy, and administration of protein mimetics and inhibitors). The screening of drugs for dyslipidemic therapy.

21 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

L2: Entry 14 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6225451 B1

TITLE: Chromosome 11-linked coronary heart disease susceptibility gene CHD1

Detailed Description Text (242):

CHD1 Fusion Proteins. Three coding sequence fragments corresponding to predicted zinc fingers 1 through 2, 1 through 8 and 3 through 8 of CHD1 were amplified from random-primed liver cDNA using PCR with Pfu enzyme (Stratagene). The primer sequences are shown in Table 11. The PCR primers incorporated restriction sites in the same translational reading frame as the same sites in the polylinker of pGEX-4T-3 (Pharmacia), a GST fusion protein expression vector. The PCR fragments are cloned into this vector using these restriction sites. The ligation reactions were transformed into DH5.alpha. cells. Protein expression from these clones was confirmed by SDS-PAGE. The pGEX 4T-3 clones were transferred to BL21 cells for large scale production of proteins. Proteins for use in the in vitro selection and gel shift experiments were synthesized as according to manufacturer's instructions (Pharmacia). For in vitro selection experiments the fusion proteins were retained on the sepharose matrix. Proteins for gel shift experiments were eluted from the glutathione-sepharose and dialyzed to remove residual glutathione. Protein concentration was estimated from SDS-polyacrylamide gels.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 15. Document ID: US 6201165 B1

L2: Entry 15 of 29

File: USPT

US-PAT-NO: 6201165

DOCUMENT-IDENTIFIER: US 6201165 B1

TITLE: Transgenic animal models for cardiac hypertrophy and methods of use thereof

DATE-ISSUED: March 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Grant; Stephen R.	Ft. Worth	TX		
Olson; Eric N.	Dallas	TX		

US-CL-CURRENT: 800/3; 435/325, 435/354, 435/366, 435/4, 435/6, 435/7.1, 435/8,
800/18

ABSTRACT:

Transgene constructs for generating transgenic animals, wherein the transgene encodes a gene product which modulates transcription of a hypertrophy-sensitive gene, are provided. Further provided are recombinant vectors comprising the transgenes of the invention. Further provided are transgenic animals generated using the transgene constructs. Further provided are enzyme-based, cell-based, and whole-animal-based assays for detecting substances having therapeutic activity toward cardiac hypertrophy. Further provided are compositions comprising substances which modulate levels of active product of a hypertrophy-sensitive gene. Further provided are methods of treating cardiac hypertrophy.

11 Claims, 54 Drawing figures

Exemplary Claim Number: 8

Number of Drawing Sheets: 23

L2: Entry 15 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6201165 B1

TITLE: Transgenic animal models for cardiac hypertrophy and methods of use thereof

Detailed Description Text (129):

As an example of protein interactive trapping, a yeast two-hybrid screen can be performed using mouse GATA4 as bait to identify potential interacting factors in the adult myocardium, as described. Molkenstin et al. (1998) Cell 93:215-228. In this example, the GATA4 bait contains amino acids 130-409 fused in-frame with the GAL4 DNA binding domain. This region of GATA4 encompasses the two zinc finger domains and is encoded within a PstI-NsiI fragment, which was cloned into a Pst I site in the pAS yeast expression vector. pAS-GATA4 was co-transformed into yeast with an adult mouse heart library that contained the GAL4 activation domain fused to random cDNAs and over 5 million primary colonies were screened for positive interactions. Approximately 100 positive yeast colonies were initially identified. From each individual colony, the activating plasmid was rescued and the cDNA insert was sequenced. Clones containing cDNA inserts in the antisense orientation or out-of-frame were discarded. The remaining clones (approximately 21) were retransformed back into yeast to test for specificity.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 16. Document ID: US 6169174 B1

L2: Entry 16 of 29

File: USPT

US-PAT-NO: 6169174

DOCUMENT-IDENTIFIER: US 6169174 B1

TITLE: Cotton plant gene

DATE-ISSUED: January 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hasegawa; Osamu	Tokyo			JP
Aotsuka; Satoshi	Tokyo			JP
Takenishi; Soichiro	Tokyo			JP
Uchimiya; Hirofumi	Kawasaki			JP

US-CL-CURRENT: 536/23.6; 435/6, 530/350, 530/370

ABSTRACT:

Nucleotide sequences of cotton plant cDNA clones randomly selected are determined. Homology search is performed between the sequences and known gene sequences registered in the data base. Thus a gene, which controls the fiber formation mechanism in cotton plant and which can be used for industrially useful improvement, is isolated from clones having homology to a gene in the data base.

10 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

L2: Entry 16 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6169174 B1

TITLE: Cotton plant gene

Brief Summary Text (15):

The present inventors prepared a cDNA library from cotton plant. The present inventors randomly selected a large amount of clones therefrom to subsequently determine their nucleotide sequences so that homology search was performed between the determined nucleotide sequences and known gene sequences included in a gene data base. In general, a method is used for cloning a gene, in which a part or all of a gene sequence having been isolated from another organism is used as a probe to perform screening from a library. However, in the present invention, homology search was performed between the nucleotide sequences of the clones randomly selected from the cDNA library and the known gene sequences in the data base. Thus it was found that a cotton plant gene was present, having high homology to a sunflower gene SF3 considered to correspond to a transcription factor specifically expressed in sunflower pollen and having two zinc finger motifs (Rachel Baltz et al., The Plant Journal, 2(5), 713-721 (1992)). Further, it was found that the gene was expressed in a large amount in fiber cells, by means of northern analysis for the cotton plant gene. Thus the present invention has been completed.

Brief Summary Text (50):

A nucleotide sequence of the gene of the present invention obtained as described above, and an amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NOs. 1 and 2 in Sequence Listing. The amino acid sequence is a novel sequence. All genes having nucleotide sequences coding for the amino acid sequence are included in the present invention. The amino acid sequence may undergo deletion, substitution, insertion, and/or addition of one or several amino acid residues provided that the characteristic of the gene of the present invention is not substantially affected. The number of the several amino acid residues may be a number to give homology of not less than 80% with respect to the amino acid sequence shown in SEQ ID NO: 2. Those including such deletion, substitution, insertion, and/or addition of one or several amino acid residues can be obtained as those having the activity of the transcription factor in fiber cells of cotton plant during cotton fiber formation, or as those expressed in a large amount in fiber cells of cotton plant during cotton fiber formation, selected from expression products of DNAs of genes modified randomly by means of an ordinary mutation

treatment or intentionally by means of the site-directed mutagenesis method. Those harboring proteins or genes having sequences partially different from the sequence shown in the Sequence Listings may exist depending on the variety of cotton plant or depending on natural mutation or the like. Such genes (variants) are also included in the gene of the present invention. These genes can be obtained as nucleotide sequences coding for the amino acid sequence shown in SEQ ID NO: 2 or a part thereof, nucleotide sequences coding for amino acid sequences subjected to mutation as described above. More specifically, these genes can be obtained as DNA hybridizable, under a stringent condition, with DNA having a sequence of nucleotide numbers of 134 to 757 in SEQ ID NO: 1 or a part thereof. By the "stringent condition" referred to herein is meant a condition under which a specific hybrid is formed, and nonspecific hybrid is not formed. It is difficult to clearly express the condition with numerical values. However, the condition is exemplified by a condition under which, nucleic acid having high homology, for example, DNA's having homology of not less than 80% are hybridized with each other, and nucleic acids having homology lower than the above are not hybridized with each other. The DNA hybridizable under the stringent condition will code for the protein comprising the polyamino acid having the zinc finger motif at a high probability.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 17. Document ID: US 6150160 A

L2: Entry 17 of 29

File: USPT

US-PAT-NO: 6150160

DOCUMENT-IDENTIFIER: US 6150160 A

TITLE: Compositions and methods of use of mammalian retrotransposons

DATE-ISSUED: November 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kazazian, Jr.; Haig H.	Baltimore	MD		
Boeke; Jef D.	Baltimore	MD		
Moran; John V.	Exton	PA		
Dombroski; Beth A.	Springfield	PA		

US-CL-CURRENT: 435/320.1; 435/455, 536/23.1, 536/23.5, 536/24.1

ABSTRACT:

The invention relates to an isolated DNAC molecule comprising a promoter P and an L1 cassette sequence comprising a core L1 retrotransposon element and methods of use thereof.

3 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 33

L2: Entry 17 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6150160 A

TITLE: Compositions and methods of use of mammalian retrotransposons

Detailed Description Text (24):

Different features of the DNAC molecule may be altered depending on the desired

application. For example, to effect site-specific insertion, as opposed to random insertion, of DNA into a host cell genome, a specific DNA binding domain may be positioned between the 5' end of ORF2 and the endonuclease domain. The specific domain may include, but is not limited to, a p53 binding domain, a zinc finger binding domain, type II endonuclease binding domain, a homeobox binding domain, and the like. The use of these domains will facilitate specific insertion of L1 retrotransposons next to genes whose expression is governed by these binding domains. Such a strategy is therefore useful for the isolation of new genes whose expression is governed by any one of the binding domains used.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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RMIC	Draw Desc	Image
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☐ 18. Document ID: US 6140466 A

L2: Entry 18 of 29

File: USPT

US-PAT-NO: 6140466

DOCUMENT-IDENTIFIER: US 6140466 A

TITLE: Zinc finger protein derivatives and methods therefor

DATE-ISSUED: October 31, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barbas, III; Carlos F.	San Diego	CA		
Gottesfeld; Joel M.	Del Mar	CA		
Wright; Peter E.	La Jolla	CA		

US-CL-CURRENT: 530/350; 435/252.3, 435/252.33, 435/320.1, 435/471, 435/69.1,
435/69.7, 530/400, 536/23.5, 536/23.6, 536/23.72

ABSTRACT:

Zinc finger proteins of the Cys.sub.2 His.sub.2 type represent a class of malleable DNA binding proteins which may be selected to bind diverse sequences. Typically, zinc finger proteins containing three zinc finger domains, like the murine transcription factor Zif268 and the human transcription factor Sp1, bind nine contiguous base pairs (bp). To create a class of proteins which would be generally applicable to target unique sites within complex genomes, the present invention provides a polypeptide linker that fuses two three-finger proteins. Two six-fingered proteins were created and demonstrated to bind 18 contiguous bp of DNA in a sequence specific fashion. Expression of these proteins as fusions to activation or repression domains allows transcription to be specifically up or down modulated within cells. Polydactyl zinc finger proteins are broadly applicable as genome-specific transcriptional switches in gene therapy strategies and the development of novel transgenic plants and animals. Such proteins are useful for inhibiting, activating or enhancing gene expression from a zinc finger-nucleotide binding motif containing promoter or other transcriptional control element, as well as a structural gene or RNA sequence.

54 Claims, 32 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 26

L2: Entry 18 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6140466 A

TITLE: Zinc finger protein derivatives and methods therefor

Brief Summary Text (18):

In yet a further embodiment, the invention provides a method for obtaining an isolated zinc finger-nucleotide binding polypeptide variant which binds to a cellular nucleotide sequence comprising identifying the amino acids in a zinc finger-nucleotide binding polypeptide that bind to a first cellular nucleotide sequence and modulate the function of the nucleotide sequence; creating an expression library encoding the polypeptide variant containing randomized substitution of the amino acids identified; expressing the library in a suitable host cell; and isolating a clone that produces a polypeptide variant that binds to a second cellular nucleotide sequence and modulates the function of the second nucleotide sequence. Preferably, the expression library encoding the polypeptide variant is a phage display library.

Drawing Description Text (125):

In general, surface membrane proteins which bind to specific effector molecules are referred to as receptors. In the present invention, antibodies are preferred receptors. Antibodies can be used to target liposomes to specific cell-surface ligands. For example, certain antigens expressed specifically on tumor cells, referred to as tumor-associated antigens (TAAs), may be exploited for the purpose of targeting antibody-zinc finger-nucleotide binding protein-containing liposomes directly to the malignant tumor. Since the zinc finger-nucleotide binding protein gene product may be indiscriminate with respect to cell type in its action, a targeted delivery system offers a significant improvement over randomly injecting non-specific liposomes. A number of procedures can be used to covalently attach either polyclonal or monoclonal antibodies to a liposome bilayer. Antibody-targeted liposomes can include monoclonal or polyclonal antibodies or fragments thereof such as Fab, or F(ab')₂, as long as they bind efficiently to an the antigenic epitope on the target cells. Liposomes may also be targeted to cells expressing receptors for hormones or other serum factors.

Drawing Description Text (126):

In another embodiment, the invention provides a method for obtaining an isolated zinc finger-nucleotide binding polypeptide variant which binds to a cellular nucleotide sequence comprising, first, identifying the amino acids in a zinc finger-nucleotide binding polypeptide that bind to a first cellular nucleotide sequence and modulate the function of the nucleotide sequence. Second, an expression library encoding the polypeptide variant containing randomized substitution of the amino acids identified in the first step is created. Third, the library is expressed in a suitable host cell, which will be apparent to those of skill in the art, and finally, a clone is isolated that produces a polypeptide variant that binds to a second cellular nucleotide sequence and modulates the function of the second nucleotide sequence. The invention also includes a zinc finger-nucleotide binding polypeptide variant produced by the method described above.

Detailed Description Text (51):

Phagemid libraries for expressing each of the randomized zinc fingers of this invention were prepared in the following procedure. To form circularized vectors containing the PCR product insert, 640 ng of the digested PCR products were admixed with 2 ug of the linearized pComb3.5 phagemid vector and ligation was allowed to proceed overnight at room temperature using 10 units of BRL ligase (Gaithersburg, Md.) in BRL ligase buffer in a reaction volume of 150 ul. Five separate ligation reactions were performed to increase the size of the phage library having randomized zinc fingers. Following the ligation reactions, the circularized DNA was precipitated at -20.degree. C. for 2 hours by the admixture of 2 ul of 20 mg/ml glycogen, 15 ul of 3M sodium acetate at pH 5.2 and 300 ul of ethanol. DNA was then pelleted by microcentrifugation at 4.degree. C. for 15 minutes. The DNA pellet was washed with cold 70% ethanol and dried under vacuum. The pellet was resuspended in 10 ul of water and transformed by electroporation into 300 ul of E. coli XL1-Blue cells to form a phage library. After transformation, to isolate phage expressing mutagenized finger 3, phage were induced as described below for subsequent panning on a hairpin oligo having the following sequence (SEQUENCE ID NO. 16):

Detailed Description Text (54):

For determining the titering colony forming units (cfu), phage (packaged phagemid) were diluted in SB and 1 ul was used to infect 50 ul of fresh (A.sub.oD600 =1) E. coli XL1-Blue cells grown in SB containing 10 ug/ml tetracycline. Phage and cells were maintained at room temperature for 15 minutes and then directly plated on LB/carbenicillin plates. The randomized zinc finger 3 library consisted of 5.times.10.sup.7 PFU total.

Detailed Description Text (61):

applied).times.100. The initial phage input ratio was determined by titering on selective plates to be approximately 10.sup.11 cfu for each round of panning. The final phage output ratio was determined by infecting two ml of logarithmic phase XL1-Blue cells as described above and plating aliquots on selective plates. From this procedure, clones were selected from the Fab library for their ability to bind to the new binding sequence oligo. The selected clones had randomized zinc finger 3 domains.

Detailed Description Text (72):

Genes encoding wild-type zinc-finger proteins were placed under the control of the Salmonella typhimurium araB promoter by insertion of a DNA fragment amplified by the polymerase chain reaction (PCR) and containing the wild-type Zif268 gene of pzif89 (Pavletich, supra) with the addition of multiple restriction sites (XhoI/SacI/ and XbaI/SpeI). The resulting plasmid vector was subsequently used for subcloning the selected zinc-finger genes for immunoscreening. In this vector the zinc finger protein is expressed as a fusion with a hemagglutinin decapeptide tag at its C-terminus which may be detected with an anti-decapeptide monoclonal antibody (FIG. 8A) (Field, et al., Mol. & Cell. Biol. 8:2159-2165, 1988). The Zif268 protein is aligned to show the conserved features of each zinc finger. The .alpha.-helices and antiparallel .beta.-sheets are indicated. Six amino-acid residues underlined in each finger sequence were randomized in library constructions. The C-terminal end of Zif268 protein was fused with a fragment containing a decapeptide tag. The position of fusion is indicated by an arrow.

Detailed Description Text (97):

Following selection for binding the native consensus or HIV-1 target sequences, functional zinc fingers were rapidly identified with an immunoscreening assay. Expression of the selected proteins in a pParaHA derivative resulted in the fusion of the mutant Zif268 proteins with a peptide tag sequence recognized by a monoclonal antibody (FIG. 8A). Binding was determined in an ELISA format using crude cell lysates. A qualitative assessment of specificity can also be achieved with this methodology which is sensitive to at least 4-fold differences in affinity. Several positive clones from each selection were sequenced and are shown in FIG. 9. The six randomized residues of finger 1 and 3 are at positions -1, 2, 3, 4, 5, and 6 in the .alpha.-helical region, and at -2, -1, 1, 2, 3, and 4 in finger 2 (FIG. 9). The three nucleotides denote the binding site used for affinity selection of each finger. Proteins studied in detail are indicated with a clone designation.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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KWAC	Draw Desc	Image
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☐ 19. Document ID: US 6117680 A

L2: Entry 19 of 29

File: USPT

US-PAT-NO: 6117680

DOCUMENT-IDENTIFIER: US 6117680 A

TITLE: Compositions and methods for regulation of transcription

DATE-ISSUED: September 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Natesan; Sridaran	Chestnut Hill	MA		
Gilman; Michael Z.	Newton	MA		

US-CL-CURRENT: 435/455; 435/235.1, 435/320.1, 435/325, 435/456, 536/23.4

ABSTRACT:

The present invention relates to novel fusion proteins which activate transcription, to nucleic acid constructs encoding the proteins and their use in the genetic engineering of cells.

62 Claims, 20 Drawing figures

Exemplary Claim Number: 14

Number of Drawing Sheets: 10

L2: Entry 19 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6117680 A

TITLE: Compositions and methods for regulation of transcription

Detailed Description Text (130):

For additional examples, information and guidance on designing, mutating, selecting, combining and characterizing DNA binding domains, see, e.g., Pomerantz J L, Wolfe S A, Pabo C O, Structure-based design of a dimeric zinc finger protein Biochemistry Jan. 27, 1998;37(4):965-970; Kim J-S and Pabo C O, Getting a Handhold on DNA: Design of Poly-zinc Finger Proteins with Femtomolar Dissociation Constants, PNAS U.S.A., Mar. 17, 1998;95(6):2812-2817; Kim J S, Pabo C O, Transcriptional repression by zinc finger peptides. Exploring the potential for applications in gene therapy, J Biol Chem 1997 Nov. 21, 1997;272(47):29795-29800; Greisman H A, Pabo C O, A general strategy for selecting high-affinity zinc finger proteins for diverse DNA target sites, Science 1997 Jan. 31, 1997;275(5300):657-661; Rebar E J, Greisman H A, Pabo C O, Phage display methods for selecting zinc finger proteins with novel DNA-binding specificities, Methods Enzymol 1996;267:129-149; Pomerantz J L, Pabo C O, Sharp P A, Analysis of homeodomain function by structure-based design of a transcription factor, Proc Natl Acad Sci U.S.A Oct. 10, 1995;92(21):9752-9756; Rebar E J, Pabo C O, Zinc finger phage: affinity selection of fingers with new DNA-binding specificities, Science, Feb. 4, 1994;263:671-673; Choo Y, Sanches-Garcia I, Klug A, In vivo repression by a site-specific DNA-binding protein designed against an oncogenic sequence, Nature, Dec. 15, 1994;372:642-645; Choo Y, Klug A, Toward a code for the interaction of zinc fingers with DNA: Selection of randomized fingers displayed on phage, PNAS U.S.A., November 1994; 91:11163-11167; Wu H, Yang W-P, Barbas C F III, Building zinc fingers by selection: toward a therapeutic application, PNAS U.S.A. January 1995; 92:344-348; Jamieson A C, Kim S-H, Wells J A, In Vitro selection of zinc fingers with altered DNA-binding specificity, Biochemistry 1994, 33:5689-5695; International patent applications WO 96/20951, WO 94/18317, WO 96/06166 and WO 95/19431; and U.S. Ser. No. 60/084819.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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RMIC	Draw Desc	Image
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☐ 20. Document ID: US 6107059 A

L2: Entry 20 of 29

File: USPT

US-PAT-NO: 6107059

DOCUMENT-IDENTIFIER: US 6107059 A

TITLE: Peptide library and screening method

DATE-ISSUED: August 22, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hart; Charles P.	Mountain View	CA		

US-CL-CURRENT: 435/69.7; 435/69.1, 435/69.8

ABSTRACT:

A random peptide library constructed by transforming host cells with a collection of recombinant vectors that encode a fusion protein comprised of a carrier protein fused to a random peptide through a proteolytic cleavage site can be used to identify ligands that bind to a receptor. The screening method results in the formation of a complex comprising the fusion protein bound to a receptor through the random peptide ligand, and the random peptide can easily be identified and analyzed by virtue of the carrier protein and associated proteolytic cleavage site.

8 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

L2: Entry 20 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6107059 A

TITLE: Peptide library and screening method

Detailed Description Text (69):

Random peptide libraries built around zinc finger scaffolding motifs may provide a rich source of lead compounds for drug discovery. A number of biologically important proteins contain the zinc finger motif. Although some of skill in the art reserve the use of the term "zinc finger" to only those motifs of the TFIIIA class, as used herein, the term encompasses any zinc coordinated motif. Zinc fingers have been implicated in protein-protein interactions, single stranded DNA binding, and RNA binding (reviewed in Berg et al., 1990, J. Biol. Chem. 265: 6513-6516, incorporated herein by reference). Members of the zinc finger protein gene family play a role in RNA processing (splicing) and DNA replication; for instance, the retroviruses, including HIV, have a variant of the zinc finger motif involved with genome packaging and virus replication. The steroid/thyroid family of nuclear receptors recognize and bind their DNA targets using a variation of the zinc finger motif (see Berg, 1989, Cell 57: 1065-1068, incorporated herein by reference). Target genes turned on by the action of the lymphokines, TNF, IL-1, and gamma-interferon, include zinc finger transcription factors believed to be responsible for mediating the next step of the inflammatory cascade (e.g. the induction of expression of cell adhesion molecules; see Opipari et al., 1990, J. Biol. Chem. 265: 14705-14708, incorporated herein by reference). Zinc finger proteins have also been shown to be associated with the neoplastic phenotype (see Tagawa et al., 1990, J. Biol. Chem. 265: 20021-20026, incorporated herein by reference).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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RMK	Draw Desc	Image
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☐ 21. Document ID: US 6077933 A

L2: Entry 21 of 29

File: USPT

US-PAT-NO: 6077933

DOCUMENT-IDENTIFIER: US 6077933 A

TITLE: Repressor Kruppel-like factor

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lee; Mu-En	Newton	MA		
McA'Nulty; Megan M.	Newton	MA		

US-CL-CURRENT: 530/300; 530/324, 530/350

ABSTRACT:

Disclosed is a novel mammalian protein designated repressor Kruppel-like factor (RKLF). RKLF is a zinc finger protein that binds to a CACCC element in DNA, thereby repressing gene transcription. An isolated DNA encoding RKLF, vectors and cells containing the DNA, and RKLF-specific antibodies are also disclosed. The RKLF DNA or protein can be introduced into the tissues of a mammal to inhibit neoplasia or hyperplasia. Also disclosed is an in vitro screening method for identifying a compound that induces RKLF gene expression.

4 Claims, 5 Drawing figures

Exemplary Claim Number: 2

Number of Drawing Sheets: 3

L2: Entry 21 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 6077933 A

TITLE: Repressor Kruppel-like factor

Detailed Description Text (18):

A cDNA probe encoding the c-terminal zinc finger region of EKLF (bp 895-1146) was generated by reverse transcription PCR (Lee et al., 1991, J. Biol. Chem. 266:16188-92; Sambrook et al., supra. The forward primer had the following sequence: 5' GAACTTTGC ACCTAAGACG CAG 3' (SEQ ID NO:3), and the sequence of the reverse primer was: 5' ACGCTTCATG TGCAGAGCTA AGTG 3' (SEQ ID NO:4). These primers were designed according to the published sequence of EKLF (Miller et al., 1993, Mol. Cell Biol. 13:2776-86). The resulting DNA fragment was labeled by random priming and used as a probe to screen a human fetal heart library in .lambda.gt11 (Clontech, Palo Alto, Calif.).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 22. Document ID: US 5928941 A

L2: Entry 22 of 29

File: USPT

US-PAT-NO: 5928941

DOCUMENT-IDENTIFIER: US 5928941 A

TITLE: Repressor kruppel-like factor

DATE-ISSUED: July 27, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lee; Mu-En	Newton	MA		
McA'Nulty; Megan M.	Newton	MA		

US-CL-CURRENT: 435/325; 435/243, 435/320.1, 435/410, 536/23.5

ABSTRACT:

Disclosed is a novel mammalian protein designated repressor Kruppel-like factor (RKLF). RKLF is a zinc finger protein that binds to a CACCC element in DNA, thereby repressing gene transcription. An isolated DNA encoding RKLF, vectors and cells containing the DNA, and RKLF-specific antibodies are also disclosed. The RKLF DNA or protein can be introduced into the tissues of a mammal to inhibit neoplasia or hyperplasia. Also disclosed is an in vitro screening method for identifying a compound that induces RKLF gene expression.

8 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

L2: Entry 22 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 5928941 A

TITLE: Repressor kruppel-like factor

Detailed Description Text (18):

A cDNA probe encoding the c-terminal zinc finger region of EKLF (bp 895-1146) was generated by reverse transcription PCR (Lee et al., 1991, J. Biol. Chem. 266:16188-92; Sambrook et al., supra. The forward primer had the following sequence: 5' GAACTTTGGC ACCTAAGAGG CAG 3' (SEQ ID NO:3), and the sequence of the reverse primer was: 5' ACGCTTCATG TGCAGAGCTA AGTG 3' (SEQ ID NO:4). These primers were designed according to the published sequence of ELF (Miller et al., 1993, Mol. Cell Biol. 13:2776-86). The resulting DNA fragment was labeled by random priming and used as a probe to screen a human fetal heart library in Xgt11 (Clontech, Palo Alto, Calif.).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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KMTC	Draw Desc	Image
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☐ 23. Document ID: US 5866325 A

L2: Entry 23 of 29

File: USPT

US-PAT-NO: 5866325

DOCUMENT-IDENTIFIER: US 5866325 A

TITLE: Methods and materials relating to the functional domains of DNA binding proteins

DATE-ISSUED: February 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sukhatme; Vikas P.	Newton Center	MA		

US-CL-CURRENT: 435/6; 435/91.2, 536/23.1, 536/24.3, 536/24.33

ABSTRACT:

Disclosed are DNA sequences encoding novel DNA binding proteins implicated in regulation of early stages of cell growth. Illustratively provided are human and mouse origin DNA sequences encoding early growth regulatory ("Egr") proteins which

include "zinc finger" regions of the type involved in DNA binding. Also disclosed is a detailed analysis of the structure and function of the early growth regulatory protein, Egr-1, delineating independent and modular activation, repression, DNA-binding, and nuclear localization activities. Also disclosed are immunological methods and materials for detection of Egr proteins and hybridization methods and materials for detection and quantification of Egr protein related nucleic acids.

16 Claims, 30 Drawing figures

Exemplary Claim Number: 2

Number of Drawing Sheets: 30

L2: Entry 23 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 5866325 A

TITLE: Methods and materials relating to the functional domains of DNA binding proteins

Detailed Description Text (160):

This localization is interesting in light of the non-random deletions [del(5q)] in human myeloid disorders (acute myelogenous leukemia) (AML), and myelodysplastic syndromes, that involve this chromosomal region. [Le Beau 1986; Dewald et al. 1985; and Van den Berghe et al. 1985]. Fifty percent of patients with therapy-related AML show chromosome 5 abnormalities (interstitial deletions or monosomy) and cytogenetic analysis of the deletions has revealed that one segment, consisting of bands 5q23-31, is absent in the malignant cells of all patients who have aberrations of chromosome 5. These data suggest that loss of a critical DNA sequence leading to hemizyosity (or homozygosity) of a recessive allele may play an important role in the pathogenesis of these disorders, a mechanism substantiated for retinoblastoma. Although genes for a number of growth factors and receptors (IL-3, GM-CSF, .beta.2-adrenergic receptor, endothelial cell growth factor, CSF-1, c-fms, pDGF receptor) are clustered in or near this region, Egr-1 (by virtue of its zinc fingers) is the only member of this group with potential transcriptional regulatory activity. It is therefore possible that its absence could lead to deregulated cell growth.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 24. Document ID: US 5773583 A

L2: Entry 24 of 29

File: USPT

US-PAT-NO: 5773583

DOCUMENT-IDENTIFIER: US 5773583 A

TITLE: Methods and materials relating to the functional domains of DNA binding proteins

DATE-ISSUED: June 30, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sukhatme; Vikas P.	Newton Center	MA		

US-CL-CURRENT: 530/358; 435/69.1, 435/69.7

ABSTRACT:

Disclosed are DNA sequences encoding novel DNA binding proteins implicated in regulation of early stages of cell growth. Illustratively provided are human and

mouse origin DNA sequences encoding early growth regulatory ("Egr") proteins which include "zinc finger" regions of the type involved in DNA binding. Also disclosed is a detailed analysis of the structure and function of the early growth regulatory protein, Egr-1, delineating independent and modular activation, repression, DNA-binding, and nuclear localization activities. Also disclosed are immunological methods and materials for detection of Egr proteins and hybridization methods and materials for detection and quantification of Egr protein related nucleic acids.

17 Claims, 30 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 30

L2: Entry 24 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 5773583 A

TITLE: Methods and materials relating to the functional domains of DNA binding proteins

Detailed Description Text (160):

This localization is interesting in light of the non-random deletions (del(5q)) in human myeloid disorders (acute myelogenous leukemia) (AML), and myelodysplastic syndromes, that involve this chromosomal region. (Le Beau, 1986; Dewald et al., 1985; and Van den Berghe et al., 1985). Fifty percent of patients with therapy-related AML show chromosome 5 abnormalities (interstitial deletions or monosomy) and cytogenetic analysis of the deletions has revealed that one segment, consisting of bands 5q23-31, is absent in the malignant cells of all patients who have aberrations of chromosome 5. These data suggest that loss of a critical DNA sequence leading to hemizyosity (or homozygosity) of a recessive allele may play an important role in the pathogenesis of these disorders, a mechanism substantiated for retinoblastoma. Although genes for a number of growth factors and receptors (IL-3, GM-CSF, .beta.2-adrenergic receptor, endothelial cell growth factor, CSF-1, c-fms, pDGF receptor) are clustered in or near this region, Egr-1 (by virtue of its zinc fingers) is the only member of this group with potential transcriptional regulatory activity. It is therefore possible that its absence could lead to deregulated cell growth.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 25. Document ID: US 5763209 A

L2: Entry 25 of 29

File: USPT

US-PAT-NO: 5763209

DOCUMENT-IDENTIFIER: US 5763209 A

TITLE: Methods and materials relating to the functional domains of DNA binding proteins

DATE-ISSUED: June 9, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sukhatme; Vikas P.	Newton Center	MA		

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 536/23.5, 536/24.31

ABSTRACT:

Disclosed are DNA sequences encoding novel DNA binding proteins implicated in

regulation of early stages of cell growth. Illustratively provided are human and mouse origin DNA sequences encoding early growth regulatory ("Egr") proteins which include "zinc finger" regions of the type involved in DNA binding. Also disclosed is a detailed analysis of the structure and function of the early growth regulatory protein, Egr-1, delineating independent and modular activation, repression, DNA-binding, and nuclear localization activities. Also disclosed are immunological methods and materials for detection of Egr proteins and hybridization methods and materials for detection and quantification of Egr protein related nucleic acids.

20 Claims, 30 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 30

L2: Entry 25 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 5763209 A

TITLE: Methods and materials relating to the functional domains of DNA binding proteins

Detailed Description Text (160):

This localization is interesting in light of the non-random deletions [del(5q)] in human myeloid disorders (acute myelogenous leukemia) (AML), and myelodysplastic syndromes, that involve this chromosomal region. [Le Beau 1986; Dewald et al. 1985; and Van den Berghe et al. 1985]. Fifty percent of patients with therapy-related AML show chromosome 5 abnormalities (interstitial deletions or monosomy) and cytogenetic analysis of the deletions has revealed that one segment, consisting of bands 5q23-31, is absent in the malignant cells of all patients who have aberrations of chromosome 5. These data suggest that loss of a critical DNA sequence leading to hemizygoty (or homozygoty) of a recessive allele may play an important role in the pathogenesis of these disorders, a mechanism substantiated for retinoblastoma. Although genes for a number of growth factors and receptors (IL-3, GM-CSF, .beta.2-adrenergic receptor, endothelial cell growth factor, CSF-1, c-fms, pDGF receptor) are clustered in or near this region, Egr-1 (by virtue of its zinc fingers) is the only member of this group with potential transcriptional regulatory activity. It is therefore possible that its absence could lead to deregulated cell growth.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 26. Document ID: US 5206152 A

L2: Entry 26 of 29

File: USPT

US-PAT-NO: 5206152

DOCUMENT-IDENTIFIER: US 5206152 A

TITLE: Cloning and expression of early growth regulatory protein genes

DATE-ISSUED: April 27, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sukhatme; Vikas P.	Chicago	IL		

US-CL-CURRENT: 435/69.1; 435/235.1, 435/252.3, 435/254.11, 435/320.1, 530/350, 530/387.9, 536/23.4, 536/23.5

ABSTRACT:

Disclosed are DNA sequences encoding novel DNA binding proteins implicated in regulation of early stages of cell growth. Illustratively provided are human and mouse origin DNA sequences encoding early growth regulatory ("Egr") proteins which include "zinc finger" regions of the type involved in DNA binding. Also disclosed are immunological methods and materials for detection of Egr proteins and hybridization methods and materials for detection and quantification of Egr protein related nucleic acids.

12 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

L2: Entry 26 of 29

File: USPT

DOCUMENT-IDENTIFIER: US 5206152 A

TITLE: Cloning and expression of early growth regulatory protein genes

Detailed Description Text (17):

This localization is interesting in light of the non-random deletions [del(5q)] in human myeloid disorders (acute myelogenous leukemia) (AML), and myelodysplastic syndromes, that involve this chromosomal region. [Le Beau et al., Science, 231, 984-987 (1986); Dewald et al., Blood, 66, 189-197 (1985); and Van den Berghe et al., Cancer Genet. Cytogenet., 17, 189-255 (1985)]. Fifty percent of patients with therapy related AML show chromosome 5 abnormalities (interstitial deletions or monosomy) and cytogenetic analysis of the deletions has revealed that one segment, consisting of bands 5q23-31, is absent in the malignant cells of all patients who have aberrations of chromosome 5. These data suggest that loss of a critical DNA sequence leading to hemizyosity (or homozygosity) of a recessive allele may play an important role in the pathogenesis of these disorders, a mechanism substantiated for retinoblastoma. Although genes for a number of growth factors and receptors (IL-3, GM-CSF, .beta..sub.2 -adrenergic receptor, endothelial cell growth factor, CSF-1, c-fms, pDGF receptor) are clustered in or near this region, Egr-1 (by virtue of its zinc fingers) is the only member of this group with potential transcriptional regulatory activity. It is therefore possible that its absence could lead to deregulated cell growth.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 27. Document ID: JP 10094392 A

L2: Entry 27 of 29

File: JPAB

Apr 14, 1998

PUB-NO: JP410094392A

DOCUMENT-IDENTIFIER: JP 10094392 A

TITLE: COTTON GENE

PUBN-DATE: April 14, 1998

INVENTOR-INFORMATION:

NAME

COUNTRY

HASEGAWA, OSAMU

AOZUKA, SATOSHI

TAKENISHI, SOICHIRO

UCHIMIYA, HIROBUMI

INT-CL (IPC): C12 N 15/09; C07 H 21/04; C07 K 14/415; C12 Q 1/68; A01 H 5/00; C12 N 1/21; C12 P 21/02

ABSTRACT:

- PROBLEM TO BE SOLVED: To obtain a new cotton gene coding a protein having a specific amino acid sequence and having a zinc finger motif, controlling the fiber-forming mechanism of cotton and capable of being expected to be useful for breed- improved cotton useful on industry.

SOLUTION: This new cotton gene codes a protein containing a polyamino acid having an amino acid sequence of the formula or the amino acid sequence of the formula wherein one or several amino acid sequences are defected, substituted, inserted or added, and having a zinc finger motif. Then new DNA is massively transcribed in fiber cells on the fiber formation of cotton, has the zinc finger motif which is one of the characteristic sequences of a transcription factor, and is used for bred-improved cotton, etc., useful on industry. The gene is obtained by making a cDNA library from cotton, and subsequently performing a homology retrieval between the base sequence of a clone randomly selected from the library and a known base sequence in a gene data base.

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L2: Entry 27 of 29

File: JPAB

Apr 14, 1998

DOCUMENT-IDENTIFIER: JP 10094392 A

TITLE: COTTON GENE

Abstract (2):

SOLUTION: This new cotton gene codes a protein containing a polyamino acid having an amino acid sequence of the formula or the amino acid sequence of the formula wherein one or several amino acid sequences are defected, substituted, inserted or added, and having a zinc finger motif. Then new DNA is massively transcribed in fiber cells on the fiber formation of cotton, has the zinc finger motif which is one of the characteristic sequences of a transcription factor, and is used for bred-improved cotton, etc., useful on industry. The gene is obtained by making a cDNA library from cotton, and subsequently performing a homology retrieval between the base sequence of a clone randomly selected from the library and a known base sequence in a gene data base.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 28. Document ID: AU 200124278 A, WO 200140798 A2

L2: Entry 28 of 29

File: DWPI

Jun 12, 2001

DERWENT-ACC-NO: 2001-374953

DERWENT-WEEK: 200154

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TITLE: Identifying genes associated with selected phenotype for research purposes, involves culturing cells transduced with nucleic acid encoding zinc finger proteins and assaying cells exhibiting selected phenotype

INVENTOR: CASE, C C; LIU, Q ; REBAR, E J

PRIORITY-DATA: 1999US-0456100 (December 6, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200124278 A	June 12, 2001		000	G01N033/50
WO 200140798 A2	June 7, 2001	E	057	G01N033/50

INT-CL (IPC): C12 N 15/10; C12 Q 1/68; G01 N 33/50

ABSTRACTED-PUB-NO: WO 200140798A

BASIC-ABSTRACT:

NOVELTY - Identifying genes (I) associated with a selected phenotype, involves providing a library (L) of nucleotide sequences encoding partially randomized zinc finger proteins (II), transducing cells with expression vectors, each comprising a sequence from (L), culturing the cells for expressing (II), assaying the cells for selected phenotype, and identifying (I), in cells exhibiting the phenotype.

DETAILED DESCRIPTION - Identifying a gene or genes (I) associated with a selected phenotype, involves providing a nucleic acid library (L) comprising nucleotide sequences that encode (II), transducing cells with expression vectors, each comprising a nucleotide sequence from (L), culturing the cells so that (II) is expressed in the cells, where (II) modulate gene expression in at least some of the cells, assaying the cells for a selected phenotype and determining whether or not the cells exhibit the selected phenotype, and identifying, in cells that exhibit the selected phenotype, the gene or genes whose expression is modulated by expression of a zinc finger protein, where gene so identified is (I).

USE - The method is useful for identifying a gene or genes associated with a selected phenotype such as the one related to cancer, nephritis, prostate hypertrophy, hematopoiesis, osteoporosis, obesity, cardiovascular disease or diabetes (claimed). The method is useful in academic laboratories, in the biotechnological industries, and in pharmaceutical, genomic, agricultural and chemical companies.

ADVANTAGE - The method is efficient and allows random inhibition or activation of uncharacterized genes that would be of great utility to scientific community. The libraries of zinc finger DNA binding proteins have the ability to regulate gene expression with high efficiency and specificity. Since the zinc finger protein provide reliable and efficient means for regulating gene expression, the libraries typically have no more than 106-107 members. This manageable library size provides high throughput applications to quickly and reliably identify genes of interest associated with any given phenotype.

L2: Entry 28 of 29

File: DWPI

Jun 12, 2001

DERWENT-ACC-NO: 2001-374953

DERWENT-WEEK: 200154

COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: Identifying genes associated with selected phenotype for research purposes, involves culturing cells transduced with nucleic acid encoding zinc finger proteins and assaying cells exhibiting selected phenotype

Basic Abstract Text (1):

NOVELTY - Identifying genes (I) associated with a selected phenotype, involves providing a library (L) of nucleotide sequences encoding partially randomized zinc finger proteins (II), transducing cells with expression vectors, each comprising a sequence from (L), culturing the cells for expressing (II), assaying the cells for selected phenotype, and identifying (I), in cells exhibiting the phenotype.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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☐ 29. Document ID: AU 726759 B, WO 9606166 A1, AU 9532291 A, EP 781331 A1, JP 10504461 W, AU 698152 B, AU 9910037 A, US 6007988 A, US 6013453 A

L2: Entry 29 of 29

File: DWPI

Nov 23, 2000

DERWENT-ACC-NO: 1996-151369
DERWENT-WEEK: 200101
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TITLE: DNA library encoding zinc finger polypeptide(s) having randomised amino acid sequence - used to inhibit oncogene expression and to regulate cell division, i.e. for use in the treatment of cancer.

INVENTOR: CHOO, Y; KLUG, A ; SANCHEZ-GARCIA, I ; GARCIA, I ; SANCHEZ GARCIA, I

PRIORITY-DATA: 1995GB-0014698 (July 18, 1995), 1994GB-0016880 (August 20, 1994), 1994GB-0022534 (November 8, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 726759 B	November 23, 2000		000	C12N015/12
WO 9606166 A1	February 29, 1996	E	087	C12N015/10
AU 9532291 A	March 14, 1996		000	C12N015/10
EP 781331 A1	July 2, 1997	E	000	C12N015/10
JP 10504461 W	May 6, 1998		107	C12N015/09
AU 698152 B	October 22, 1998		000	C12N015/10
AU 9910037 A	April 22, 1999		000	C12N015/12
US 6007988 A	December 28, 1999		000	C12Q001/68
US 6013453 A	January 11, 2000		000	C12Q001/68

INT-CL (IPC): A61 K 38/00; A61 K 48/00; C07 H 21/04; C07 K 14/47; C12 N 15/09; C12 N 15/10; C12 N 15/12; C12 N 15/62; C12 Q 1/68

ABSTRACTED-PUB-NO: US 6007988A

BASIC-ABSTRACT:

A library of DNA sequences, each sequence encoding at least one zinc finger binding motif for display on a viral particle and having random allocation of amino acids at positions -1, +2, +3 and +6 and at least at one of positions +1, +5 and +8.

USE - The zinc finger polypeptide may be used to alter the expression of a gene of interest by designing the zinc finger polypeptides to bind to a structural and/or regulatory region of the gene of interest. The zinc finger polypeptide may comprise the nuclear localisation signal from the large T antigen of SV40 and may therefore be used to inhibit cell division. The zinc finger polypeptides may therefore be used in the treatment of cancer (claimed). The zinc finger polypeptides may also be used to modify a nucleic acid sequence, by binding the zinc finger polypeptide to the target DNA and removing the zinc finger polypeptide and the specifically bound nucleic acid sequence from the rest of the sample. The zinc finger polypeptides may be designed to inhibit the expression of a disease associated gene, pref. an oncogene, esp. a ras or a BCR-ABL fusion oncogene (claimed). Kits for the production of the zinc finger polypeptides are also included within the scope of the invention.

ABSTRACTED-PUB-NO:

US 6013453A EQUIVALENT-ABSTRACTS:

A library of DNA sequences, each sequence encoding at least one zinc finger binding motif for display on a viral particle and having random allocation of amino acids at positions -1, +2, +3 and +6 and at least at one of positions +1, +5 and +8.

USE - The zinc finger polypeptide may be used to alter the expression of a gene of interest by designing the zinc finger polypeptides to bind to a structural and/or regulatory region of the gene of interest. The zinc finger polypeptide may comprise the nuclear localisation signal from the large T antigen of SV40 and may therefore be used to inhibit cell division. The zinc finger polypeptides may therefore be used

- in the treatment of cancer (claimed). The zinc finger polypeptides may also be used
- to modify a nucleic acid sequence, by binding the zinc finger polypeptide to the
- target DNA and removing the zinc finger polypeptide and the specifically bound nucleic acid sequence from the rest of the sample. The zinc finger polypeptides may be designed to inhibit the expression of a disease associated gene, pref. an oncogene, esp. a ras or a BCR-ABL fusion oncogene (claimed). Kits for the production of the zinc finger polypeptides are also included within the scope of the invention.

A library of DNA sequences, each sequence encoding at least one zinc finger binding motif for display on a viral particle and having random allocation of amino acids at positions -1, +2, +3 and +6 and at least at one of positions +1, +5 and +8.

USE - The zinc finger polypeptide may be used to alter the expression of a gene of interest by designing the zinc finger polypeptides to bind to a structural and/or regulatory region of the gene of interest. The zinc finger polypeptide may comprise the nuclear localisation signal from the large T antigen of SV40 and may therefore be used to inhibit cell division. The zinc finger polypeptides may therefore be used in the treatment of cancer (claimed). The zinc finger polypeptides may also be used to modify a nucleic acid sequence, by binding the zinc finger polypeptide to the target DNA and removing the zinc finger polypeptide and the specifically bound nucleic acid sequence from the rest of the sample. The zinc finger polypeptides may be designed to inhibit the expression of a disease associated gene, pref. an oncogene, esp. a ras or a BCR-ABL fusion oncogene (claimed). Kits for the production of the zinc finger polypeptides are also included within the scope of the invention.

WO 9606166A

L2: Entry 29 of 29

File: DWPI

Nov 23, 2000

DERWENT-ACC-NO: 1996-151369

DERWENT-WEEK: 200101

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TITLE: DNA library encoding zinc finger polypeptide(s) having randomised amino acid sequence - used to inhibit oncogene expression and to regulate cell division, i.e. for use in the treatment of cancer.

Standard Title Terms (1):

DNA LIBRARY ENCODE ZINC FINGER POLYPEPTIDE RANDOM AMINO ACID SEQUENCE INHIBIT ONCOGENIC EXPRESS REGULATE CELL DIVIDE TREAT CANCER

Full Title Citation Front Review Classification Date Reference Sequences Attachments

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Term	Documents
CELL.DWPI,EPAB,JPAB,USPT,PGPB.	794166
CELLS.DWPI,EPAB,JPAB,USPT,PGPB.	534476
VIVO.DWPI,EPAB,JPAB,USPT,PGPB.	100160
VIVOES	0
VIVOS.DWPI,EPAB,JPAB,USPT,PGPB.	32
VIVOE	0
(1 SAME (VIVO OR CELL)).USPT,PGPB,JPAB,EPAB,DWPI.	29
(L1 SAME (CELL OR VIVO)).USPT,PGPB,JPAB,EPAB,DWPI.	29

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NEWS 5 Feb 19 Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6 Mar 08 Gene Names now available in BIOSIS
NEWS 7 Mar 22 TOXLIT no longer available
NEWS 8 Mar 22 TRCTHERMO no longer available
NEWS 9 Mar 28 US Provisional Priorities searched with P in CA/CAplus and USPATFULL
NEWS 10 Mar 28 LIPINSKI/CALC added for property searching in REGISTRY
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NEWS 12 Apr 08 "Ask CAS" for self-help around the clock
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NEWS 17 Apr 22 BIOSIS Gene Names now available in TOXCENTER
NEWS 18 Apr 22 Federal Research in Progress (FEDRIP) now available
NEWS 19 Jun 03 New e-mail delivery for search results now available
NEWS 20 Jun 10 MEDLINE Reload
NEWS 21 Jun 10 PCTFULL has been reloaded
NEWS 22 Jul 02 FOREGE no longer contains STANDARDS file segment
NEWS 23 Jul 19 NTIS to be reloaded July 28, 2002
NEWS 24 Jul 22 USAN to be reloaded July 28, 2002; saved answer sets no longer valid

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=> s zinc finger

L1 13039 ZINC FINGER

=> s l1 and random?

L2 214 L1 AND RANDOM?

=> s l2 and (cell or vivo)

L3 108 L2 AND (CELL OR VIVO)

=> s l2 and (screen or assay) and cell

L4 31 L2 AND (SCREEN OR ASSAY) AND CELL

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L5 ANSWER 1 OF 21 MEDLINE

DUPLICATE 1

AN 2002303142 MEDLINE

DN 22022021 PubMed ID: 12024037

TI A Kruppel-associated box-**zinc finger** protein, NT2,
represses **cell**-type-specific promoter activity of the alpha
2(XI) collagen gene.

AU Tanaka Kazuhiro; Tsumaki Noriyuki; Kozak Christine A; Matsumoto Yoshihiro;
Nakatani Fumihiko; Iwamoto Yukihide; Yamada Yoshihiko

CS Craniofacial Developmental Biology and Regeneration Branch, National
Institute of Dental and Craniofacial Research, National Institutes of
Health, Bethesda, Maryland 20892, USA.

SO MOLECULAR AND CELLULAR BIOLOGY, (2002 Jun) 22 (12) 4256-67.
Journal code: 8109087. ISSN: 0270-7306.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200206

ED Entered STN: 20020605

Last Updated on STN: 20020625

Entered Medline: 20020624

AB Type XI collagen is composed of three chains, alpha 1(XI), alpha 2(XI),
and alpha 3(XI), and plays a critical role in the formation of cartilage
collagen fibrils and in skeletal morphogenesis. It was previously reported
that the -530-bp promoter segment of the alpha 2(XI) collagen gene
(Col11a2) was sufficient for cartilage-specific expression and that a
24-bp sequence from this segment was able to switch promoter activity from
neural tissues to cartilage in transgenic mice when this sequence was
placed in the heterologous neurofilament light gene (NFL) promoter. To
identify a protein factor that bound to the 24-bp sequence of the Col11a2
promoter, we screened a mouse limb bud cDNA expression library in the
yeast one-hybrid screening system and obtained the cDNA clone NT2.
Sequence analysis revealed that NT2 is a **zinc finger**
protein consisting of a Kruppel-associated box (KRAB) and is a homologue
of human FPM315, which was previously isolated by **random** cloning

and sequencing. The KRAB domain has been found in a number of **zinc finger** proteins and implicated as a transcriptional repression domain, although few target genes for KRAB-containing **zinc finger** proteins has been identified. Here, we demonstrate that NT2 functions as a negative regulator of Col11a2. In situ hybridization analysis of developing mouse cartilage showed that NT2 mRNA is highly expressed by hypertrophic chondrocytes but is minimally expressed by resting and proliferating chondrocytes, in an inverse correlation with the expression patterns of Col11a2. Gel shift **assays** showed that NT2 bound a specific sequence within the 24-bp site of the Col11a2 promoter. We found that Col11a2 promoter activity was inhibited by transfection of the NT2 expression vector in RSC **cells**, a chondrosarcoma **cell** line. The expression vector for mutant NT2 lacking the KRAB domain failed to inhibit Col11a2 promoter activity. These results demonstrate that KRAB-**zinc finger** protein NT2 inhibits transcription of its physiological target gene, suggesting a novel regulatory mechanism of cartilage-specific expression of Col11a2.

L5 ANSWER 2 OF 21 MEDLINE DUPLICATE 2
 AN 2002112300 MEDLINE
 DN 21831100 PubMed ID: 11842116
 TI Neuroendocrine differentiation factor, IA-1, is a transcriptional repressor and contains a specific DNA-binding domain: identification of consensus IA-1 binding sequence.
 AU Breslin Mary B; Zhu Min; Notkins Abner L; Lan Michael S
 CS Research Institute for Children, Children's Hospital, Department of Pediatrics, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA.
 SO NUCLEIC ACIDS RESEARCH, (2002 Feb 15) 30 (4) 1038-45.
 CY England: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200203
 ED Entered STN: 20020215
 Last Updated on STN: 20020305
 Entered Medline: 20020304
 AB A novel cDNA, insulinoma-associated antigen-1 (IA-1), containing five **zinc-finger** DNA-binding motifs, was isolated from a human insulinoma subtraction library. IA-1 expression is restricted to fetal but not adult pancreatic and brain tissues as well as tumors of neuroendocrine origin. Using various GAL4 DNA binding domain (DBD)/IA-1 fusion protein constructs, we demonstrated that IA-1 functions as a transcriptional repressor and that the region between amino acids 168 and 263 contains the majority of the repressor activity. Using a selected and amplified **random** oligonucleotide binding **assay** and bacterially expressed GST-IA-1DBD fusion protein (257-510 a.a.), we identified the consensus IA-1 binding sequence, TG/TC/TC/TT/AGGGGG/TCG/A. Further experiments showed that **zinc-fingers** 2 and 3 of IA-1 are sufficient to demonstrate transcriptional activity using an IA-1 consensus site containing a reporter construct. A database search with the consensus IA-1 binding sequence revealed target sites in a number of pancreas- and brain-specific genes consistent with its restricted expression pattern. The most significant matches were for the 5'-flanking regions of IA-1 and NeuroD/beta2 genes. Co-transfection of **cells** with either the full-length IA-1 or hEgr-1AD/IA-1DBD construct and IA-1 or NeuroD/beta2 promoter/CAT construct modulated CAT activity. These findings suggest that the IA-1 protein may be auto-regulated and play a role in pancreas and neuronal development, specifically in the regulation of the NeuroD/beta2 gene.

L5 ANSWER 3 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 AN 2002:233085 BIOSIS
 DN PREV200200233085

TI Identification of a minimal HIV-1 Gag domain sufficient for self-association.

AU Zabransky, Ales; Hunter, Eric; Sakalian, Michael (1)

CS (1) Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, 940 Stanton L. Young Boulevard, Oklahoma City, OK, 73190: mike-sakalian@ouhsc.edu USA

SO Virology, (March 1, 2002) Vol. 294, No. 1, pp. 141-150.
<http://www.academicpress.com/virology>. print.
 ISSN: 0042-6822.

DT Article

LA English

AB Gag polyprotein precursors play an essential role in the assembly of the HIV particle by polymerizing into a spherical shell at the plasma membrane. In order to define the domains within Gag responsible for this homotypic interaction, we have coupled the technology of the yeast two-hybrid system with the technology of a gene-based, semirandom library. By this method, we have identified a minimal region of Gag capable of efficient self-interaction. This region consists of the N-terminal portion of the nucleocapsid protein (NC), including the first **zinc finger** and the previously described interaction, or I, domain. In parallel with this **randomized** approach, individual HIV Gag domains, and combinations of these domains, were tested for potential homotypic and heterotypic interactions in the yeast two-hybrid system. Consistent with the results from the semirandom library **screen**, only combinations of species containing NC were strongly interacting.

L5 ANSWER 4 OF 21 MEDLINE

AN 2001112321 MEDLINE

DN 20584911 PubMed ID: 11154279

TI Sequence-specific transcriptional repression by KS1, a multiple-**zinc-finger**-Kruppel-associated box protein.

AU Gebelein B; Urrutia R

CS Department of Molecular Neuroscience, Mayo Clinic, Rochester, Minnesota 55905, USA.

NC DK5660 (NIDDK)

SO MOLECULAR AND CELLULAR BIOLOGY, (2001 Feb) 21 (3) 928-39.
 Journal code: 8109087. ISSN: 0270-7306.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200102

ED Entered STN: 20010322
 Last Updated on STN: 20010322
 Entered Medline: 20010208

AB The vertebrate genome contains a large number of Kruppel-associated box-**zinc finger** genes that encode 10 or more C(2)-H(2) **zinc finger** motifs. Members of this gene family have been proposed to function as transcription factors by binding DNA through their **zinc finger** region and repressing gene expression via the KRAB domain. To date, however, no Kruppel-associated box-**zinc finger** protein (KRAB-ZFP) and few proteins with 10 or more **zinc finger** motifs have been shown to bind DNA in a sequence-specific manner. Our laboratory has recently identified KS1, a member of the KRAB-ZFP family that contains 10 different C(2)-H(2) **zinc finger** motifs, 9 clustered at the C terminus with an additional **zinc finger** separated by a short linker region. In this study, we used a **random** oligonucleotide binding **assay** to identify a 27-bp KS1 binding element (KBE). Reporter **assays** demonstrate that KS1 represses the expression of promoters containing this DNA sequence. Deletion and site-directed mutagenesis reveal that KS1 requires nine C-terminal **zinc fingers** and the KRAB domain for transcriptional repression through the KBE site, whereas the isolated **zinc finger** and linker region are dispensable for this function.

Additional biochemical **assays** demonstrate that the KS1 KRAB domain interacts with the KAP-1 corepressor, and mutations that abolish this interaction alleviate KS1-mediated transcriptional repression. Thus, this study provides the first direct evidence that a KRAB-ZFP binds DNA to regulate gene expression and provides insight into the mechanisms used by multiple-**zinc-finger** proteins to recognize DNA sequences.

L5 ANSWER 5 OF 21 MEDLINE
 AN 2001359883 MEDLINE
 DN 21313905 PubMed ID: 11419965
 TI Improvement of RNA fingerprinting efficiency for the analysis of differential gene expression in human cardiac macro- and microvascular endothelial **cells**.
 AU Bongrazio M; Grafe M; Pries A R; Gaehtgens P; Zakrzewicz A
 CS Department of Physiology, Freie Universitat Berlin, Arnimallee 22, 14195 Berlin, Germany.. mbon@zedat.fu-berlin.de
 SO PHARMACOLOGICAL RESEARCH, (2001 Jun) 43 (6) 553-60.
 Journal code: 8907422. ISSN: 1043-6618.
 CY England: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200109
 ED Entered STN: 20011001
 Last Updated on STN: 20011001
 Entered Medline: 20010927
 AB RNA fingerprinting by arbitrarily primed PCR (RAP-PCR) is a powerful tool to **screen** differential gene expression. However, PCR-based screening techniques show a high incidence of false positive results (40-90%). In order to increase the efficiency and feasibility of RAP-PCR, the original protocol was modified and applied to analyse differential gene expression in human coronary macro- (HCEC) and microvascular (HCMEC) endothelial **cells**. The major modifications introduced were: (i) the use of two primers for PCR amplification, instead of reverse-transcription primer alone; (ii) the use of three cycles at low stringency followed by further amplification at high stringency; (iii) optimization of amplification cycle number, template amount, and concentration of primers, dNTP, Mg(2+); (iv) detection of fingerprints by silver staining; and (v) direct sequencing using RAP-PCR primers. Analysis of untreated and TNF alpha -stimulated (100 U ml(-1) for 1, 4, and 24 h) HCEC and HCMEC displayed 11 differentially expressed products by 18 primer combinations. Confirmation of results by RT-PCR showed that the rate of false positives attributable to our screening method was less than 20%. Among detected RAP-PCR products, the expression of Mn-superoxide dismutase, A20 **zinc finger** protein, and three novel genes (A/a, 4/d, 7/c) was more strongly modulated by TNF in HCEC than HCMEC. A further novel gene (B/e) was strongly expressed in HCMEC while only barely detectable in HCEC. In conclusion, modification of RAP-PCR strongly reduced the incidence of false positives, eliminated a radioactive requirement, and allowed sequencing without prior cloning, supplying an improved technology able to identify new differentially expressed genes between macro- and microvascular endothelial **cells**.

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L5 ANSWER 6 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 AN 2002:241352 BIOSIS
 DN PREV200200241352
 TI Correlation of gene expression between FKLF/FKLF-2 and gamma globin gene in individual BFU-E colonies of a patient with anemia.
 AU Aoki, Etsuko (1); Asano, Haruhiko (1); Hatano, Sonoko (1); Stamatoyannopoulos, George; Kinoshita, Tomohiro (1); Murate, Takashi; Saito, Hidehiko (1)
 CS (1) 1st Dept of Int Med, Nagoya University School of Medicine, Nagoya

Japan
SO Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 500a.
<http://www.bloodjournal.org/>. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology,
Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DT Conference
LA English
AB FKLf and FKLf-2, Kruppel-like **zinc finger** proteins,
were cloned from human fetal erythroid **cells** and mouse yolk sac
cells, respectively. They activate predominantly gamma globin gene
promoter in transient expression **assay** (Asano et. al, Mol.
Cell. Biol, 19, 1999, Blood, 95, 2000). To determine whether FKLf
and FKLf-2 play a role in the gamma globin gene expression in vivo, we
analyzed the mRNA expression levels of FKLf, FKLf-2 and gamma globin genes
in individual BFU-E colonies using real time RT-PCR. The BFU-E colonies
were formed from bone marrow mononuclear **cells** of a patient with
MDS who showed increased F **cells** in his peripheral blood smear
by the acid elution method. Seventy BFU-E colonies were plucked.
Subsequently, total RNA was extracted from individual colonies and cDNA
was synthesized using **random** hexamers. We analyzed the mRNA
expression levels of FKLf/FKLf-2 and gamma globin genes of each colony
using by real time PCR. The gene amplification of the PCR was specific
since primer sets used in this study generated a unique band by agarose
gel electrophoresis. Considering the difference of the quantity and the
quality of RNA used, the mRNA expression levels of those genes were
normalized to glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA
expression level by the real time PCR. Sixty-two colonies were evaluable.
Correlation coefficients (r values) between FKLf/FKLf-2 and gamma globin
gene expressions normalized to GAPDH expression were determined. As to
FKLf, it was 0.69, while it was 0.41 between FKLf-2 and gamma globin.
These results suggest that the expression of FKLf, but not FKLf-2, well
correlates with gamma globin gene expression in vivo. To test the
relationship between FKLf and gamma globin gene expression further, we
targeted one allele of the FKLf gene in K562 **cells**. When
analyzed with real time RT-PCR, four FKLf+/- K562 clones somehow, although
paradoxically, expressed FKLf mRNA at a higher level (2.7 times higher, in
average), and those clones also showed higher gamma globin mRNA expression
(2.8 times higher, in average). Thus, also in this system, the correlation
between FKLf and gamma globin gene expression was observed. Our results
strongly suggest that FKLf plays a role in the gamma globin gene
expression in vivo.

L5 ANSWER 7 OF 21 MEDLINE
AN 2001650093 MEDLINE
DN 21559722 PubMed ID: 11702697
TI Constructing and expression of three **zinc-fingers**
peptide with specific DNA recognition property in Escherichia coli.
AU Zhang S X; Ma Q J; Zhao Z H
CS Beijing Institute of Biotechnology, Beijing 100850, China.
SO SHENG WU KUNG CH ENG HSUEH PAO, (2001 Jul) 17 (4) 406-9.
Journal code: 9426463. ISSN: 1000-3061.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA Chinese
FS Priority Journals
EM 200112
ED Entered STN: 20011113
Last Updated on STN: 20020123
Entered Medline: 20011205
AB For investigating the DNA binding property of classical **zinc**
finger protein Zif268, an in vivo transcription interference
experiment was once utilized to develop a genetic selection **assay**
. By screening a library in which the key amino acids of the third
zinc finger from Zif268 were **randomized**, some

single fingers with new binding specificity were obtained. In this study, by combining the single fingers, two three-finger peptides cDNA ZF123 and 2ZF123 were constructed by an over-lap PCR technique using the DNA binding domain of Zif268 as the template. After three times PCR, the products were inserted into pUC18 for cloning. The ZF123 and 2ZF123 cDNA were also inserted into pGEX-2T for expression in *Escherichia coli* after sequencing confirmation. The result showed that the three-finger peptides were expressed at a high level in *E. coli* JM109. The fusion protein GST-ZF123/2ZF123 have the relative molecular weight of 34.0 kD and consisted about 20% of the total soluble cell protein as detected by SDS-PAGE. After supersonic treatment, the soluble part of the bacterial extract was purified. After two additional thrombin cleavage and Sepharose 4B affinity purification steps, the free three-fingers peptide proteins were also obtained. The construction and obtaining of the three-fingers peptide cDNA and its products will facilitate the in vivo and in vitro DNA binding specificity study and the design of the hybrid transcription factors.

L5 ANSWER 8 OF 21 MEDLINE DUPLICATE 3
 AN 2001360421 MEDLINE
 DN 21317063 PubMed ID: 11424210
 TI In silico mining of EST databases for novel pre-implantation embryo-specific **zinc finger** protein genes.
 AU Choo K B; Chen H H; Cheng W T; Chang H S; Wang M
 CS Recombinant DNA Laboratory, Department of Medical Research and Education, Veterans General Hospital-Taipei, Shih-Pai, Taipei, Taiwan..
 kcbhu@vghtpe.gov.tw
 SO MOLECULAR REPRODUCTION AND DEVELOPMENT, (2001 Jul) 59 (3) 249-55.
 Journal code: 8903333. ISSN: 1040-452X.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 OS GENBANK-AA422810; GENBANK-AA549412; GENBANK-AA666887
 EM 200110
 ED Entered STN: 20011029
 Last Updated on STN: 20011029
 Entered Medline: 20011025
 AB Progress in the understanding of early mammalian embryo development has been severely hampered by scarcity of study materials. To circumvent such a constraint, we have developed a strategy that involves a combination of in silico mining of new genes from expressed sequence tags (EST) databases and rapid determination of expression profiles of the dbEST-derived genes using a PCR-based **assay** and a panel of cDNA libraries derived from different developmental stages and somatic tissues. We demonstrate that in a **random** sample of 49 independent dbEST-derived **zinc finger** protein genes mined from a mouse embryonic 2-cell cDNA library, more than three-quarters of these genes are novel. Examination of characteristics of the human orthologues derived from these mouse genes reveals that many of them are associated with human malignancies. Expression studies have further led to the identification of three novel genes that are exclusively expressed in mouse embryos before or up to the 8-cell stage. Two of the genes, designated 2czf45 and 2czf48 (2czf for 2-cell **zinc finger**), are **zinc finger** protein genes coding for a RBCC protein with a RFP domain and a protein with three C2H2 fingers, respectively. The third gene, designated 2cpoz56, codes for a protein with a POZ domain that is often associated with **zinc finger** proteins. These three genes are candidate genes for regulatory or other functions in early embryogenesis. The strategy described in this report should generally be applicable to rapid and large-scale mining of other classes of rare genes involved in other biological and pathological processes. Mol. Reprod. Dev. 59:249-255, 2001.
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L5 ANSWER 9 OF 21 MEDLINE DUPLICATE 4
 AN 2000092855 MEDLINE
 DN 20092855 PubMed ID: 10625627
 TI The kappaB and V(D)J recombination signal sequence binding protein KRC regulates transcription of the mouse metastasis-associated gene S100A4/mts1.
 AU Hjelmsoe I; Allen C E; Cohn M A; Tulchinsky E M; Wu L C
 CS Danish Cancer Society, Department of Molecular Cancer Biology, Strandboulevarden 49, DK-2100 Copenhagen, Denmark.
 NC GM48798 (NIGMS)
 P30 CA16058 (NCI)
 T32
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jan 14) 275 (2) 913-20.
 Journal code: 2985121R. ISSN: 0021-9258.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200002
 ED Entered STN: 20000309
 Last Updated on STN: 20000824
 Entered Medline: 20000218
 AB A kappaB-like sequence, Sb, is integral to the composite enhancer located in the first intron of the metastasis-associated gene, S100A4/mts1. Oligonucleotides containing this sequence form three specific complexes with nuclear proteins prepared from S100A4/mts1-expressing CSML100 adenocarcinoma **cells**. Protein studies show the Sb-interacting complexes include NF-kappaB/Rel proteins, p50.p50 and p50.p65 dimers. Additionally, the Sb sequence was bound by an unrelated approximately 200-kDa protein, p200. Site-directed mutagenesis in conjunction with transient transfections indicate that p200, but not the NF-kappaB/Rel proteins, transactivates S100A4/mts1. To identify candidate genes for p200, double-stranded DNA probes containing multiple copies of Sb were used to **screen** a **randomly** primed lambda_{gt}11 cDNA expression library made from CSML100 poly(A)(+) RNA. Two clones corresponding to the DNA-binding proteins KRC and Alf1 were identified. KRC encodes a large **zinc finger** protein that binds to the kappaB motif and to the signal sequences of V(D)J recombination. In vitro DNA binding **assays** using bacterially expressed KRC fusion proteins, demonstrate specific binding of KRC to the Sb sequence. In addition, introduction of KRC expression vectors into mammalian **cells** induces expression of S100A4/mts1 and reporter genes driven by S100A4/mts1 gene regulatory sequences. These data indicate that KRC positively regulates transcription of S100A4/mts1.

L5 ANSWER 10 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 AN 2000:172550 BIOSIS
 DN PREV200000172550
 TI Characterization of Drosophila OVO protein DNA binding specificity using **random** DNA oligomer selection suggests **zinc finger** degeneration.
 AU Lee, Sanggyu; Garfinkel, Mark D. (1)
 CS (1) Division of Biology, Illinois Institute of Technology, Chicago, IL, 60616 USA
 SO Nucleic Acids Research., (Feb. 1, 2000) Vol. 28, No. 3, pp. 826-834.
 ISSN: 0305-1048.
 DT Article
 LA English
 SL English
 AB The Drosophila melanogaster ovo locus codes for several tissue- and stage-specific proteins that all possess a common C-terminal array of four C2H2 **zinc fingers**. Three fingers conform to the motif framework and are evolutionarily conserved; the fourth diverges considerably. The ovo genetic function affects germ **cell** viability, sex identity and oogenesis, while the overlapping svb function

is a key selector for epidermal structures under the control of wnt and EGF receptor signaling. We isolated synthetic DNA oligomers bound by the OVO **zinc finger** array from a high complexity starting population and derived a statistically significant 9 bp long DNA consensus sequence, which is nearly identical to a consensus derived from several *Drosophila* genes known or suspected of being regulated by the ovo function in vivo. The DNA consensus recognized by *Drosophila* OVO protein is atypical for **zinc finger** proteins in that it does not conform to many of the 'rules' for the interaction of amino acid contact residues and DNA bases. Additionally, our results suggest that only three of the OVO **zinc fingers** contribute to DNA-binding specificity.

L5 ANSWER 11 OF 21 MEDLINE
 AN 2000110781 MEDLINE
 DN 20110781 PubMed ID: 10646861
 TI PLAG1, the main translocation target in pleomorphic adenoma of the salivary glands, is a positive regulator of IGF-II.
 AU Voz M L; Agten N S; Van de Ven W J; Kas K
 CS Laboratory for Molecular Oncology, Center for Human Genetics, University of Leuven, Belgium.. marianne.voz@med.kuleuven.ac.be
 SO CANCER RESEARCH, (2000 Jan 1) 60 (1) 106-13.
 Journal code: 2984705R. ISSN: 0008-5472.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200002
 ED Entered STN: 20000218
 Last Updated on STN: 20000218
 Entered Medline: 20000204
 AB PLAG1, a novel developmentally regulated C2H2 **zinc finger** gene, is consistently rearranged and overexpressed in pleomorphic adenomas of the salivary glands with 8q12 translocations. In this report, we show that PLAG1 is a nuclear protein that binds DNA in a specific manner. The consensus PLAG1 binding site is a bipartite element containing a core sequence, GRGGC, and a G-cluster, RGGK, separated by seven **random** nucleotides. DNA binding is mediated mainly via three of the seven **zinc fingers**, with fingers 6 and 7 interacting with the core and finger 3 with the G-cluster. In transient transactivation **assays**, PLAG1 specifically activates transcription from its consensus DNA binding site, indicating that PLAG1 is a genuine transcription factor. Potential PLAG1 binding sites were found in the promoter 3 of the human insulin-like growth factor II (IGF-II) gene. We show that PLAG1 binds IGF-II promoter 3 and stimulates its activity. Moreover, IGF-II transcripts derived from the P3 promoter are highly expressed in salivary gland adenomas overexpressing PLAG1. In contrast, they are not detectable in adenomas without abnormal PLAG1 expression nor in normal salivary gland tissue. This indicates a perfect correlation between PLAG1 and IGF-II expression. All of these results strongly suggest that IGF-II is one of the PLAG1 target genes, providing us with the first clue for understanding the role of PLAG1 in salivary gland tumor development.

L5 ANSWER 12 OF 21 MEDLINE DUPLICATE 5
 AN 1999367495 MEDLINE
 DN 99367495 PubMed ID: 10438541
 TI Analysis of estrogen response element binding by genetically selected steroid receptor DNA binding domain mutants exhibiting altered specificity and enhanced affinity.
 AU Chusacultanachai S; Glenn K A; Rodriguez A O; Read E K; Gardner J F; Katzenellenbogen B S; Shapiro D J
 CS Department of Biochemistry, University of Illinois, Urbana, Illinois 61801, USA.
 NC CA 60514 (NCI)

GM 28717 (NIGMS)
 HD-16720 (NICHD)
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Aug 13) 274 (33) 23591-8.
 Journal code: 2985121R. ISSN: 0021-9258.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199909
 ED Entered STN: 19990913
 Last Updated on STN: 19990913
 Entered Medline: 19990901
 AB To analyze the role of amino acids in the steroid receptor DNA binding domain (DBD) recognition helix in binding of the receptor to the estrogen response element (ERE), we adapted the powerful P22 challenge phage selection system for use with a vertebrate protein. We used the progesterone receptor DNA binding domain and selected for mutants that gained the ability to bind to the ERE. We used a mutagenesis protocol based on degenerate oligonucleotides to create a large and diverse pool of mutants in which 10 nonconsensus amino acids in the DNA recognition helix of the progesterone receptor DNA binding domain were **randomly** mutated. After a single cycle of modified P22 challenge phage selection, 37 mutant proteins were identified, all of which lost the ability to bind to the progesterone response element. In gel mobility shift **assays**, approximately 70% of the genetically selected mutants bound to the consensus ERE with a >4-fold higher affinity than the naturally occurring estrogen receptor DBD. In the P-box region of the DNA recognition helix, the selected mutants contained the amino acids found in the wild-type estrogen receptor DBD, as well as other amino acid combinations seen in naturally occurring steroid/nuclear receptors that bind the aGGTCA half-site. We also obtained high affinity DBDs with Trp(585) as the first amino acid of the P-box, although this is not found in the known steroid/nuclear receptors. In the linker region between the two **zinc fingers**, G597R was by far the most common mutation. In transient transfections in mammalian **cells** using promoter interference **assays**, the mutants displayed enhanced affinity for the ERE. When linked to an activation domain, the transfected mutants activated transcription from ERE-containing reporter genes. We conclude that the P-box amino acids can display considerable variation and that the little studied linker amino acids play an important role in determining affinity for the ERE. This work also demonstrates that the P22 challenge phage genetic selection system, modified for use with a mammalian protein, provides a novel, single cycle selection for steroid/nuclear receptor DBDs with altered specificity and greatly enhanced affinity for their response elements.

L5 ANSWER 13 OF 21 MEDLINE DUPLICATE 6
 AN 1998444968 MEDLINE
 DN 98444968 PubMed ID: 9773984
 TI Characterization of a nuclear deformed epidermal autoregulatory factor-1 (DEAF-1)-related (NUDR) transcriptional regulator protein.
 AU Huggenvik J I; Michelson R J; Collard M W; Ziemba A J; Gurley P; Mowen K A
 CS Department of Physiology, Southern Illinois University School of Medicine, Carbondale 62901-6523, USA.. jhuggenvik@som.siu.edu
 NC HD-31613 (NICHD)
 HD-32484 (NICHD)
 SO MOLECULAR ENDOCRINOLOGY, (1998 Oct) 12 (10) 1619-39.
 Journal code: 8801431. ISSN: 0888-8809.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 OS GENBANK-AF049459; GENBANK-AF049460; GENBANK-AF049461; GENBANK-AF055884
 EM 199812
 ED Entered STN: 19990115

Last Updated on STN: 20000303

Entered Medline: 19981221

AB A monkey kidney cDNA that encodes a nuclear regulatory factor was identified by expression and affinity binding to a synthetic retinoic acid response element (RARE) and was used to isolate human placental and rat germ cell cDNAs by hybridization. The cDNAs encode a 59-kDa protein [nuclear DEAF-1-related (NUDR)] which shows sequence similarity to the Drosophila Deformed epidermal autoregulatory factor-1 (DEAF-1), a nonhomeodomain cofactor of embryonic Deformed gene expression. Similarities to other proteins indicate five functional domains in NUDR including an alanine-rich region prevalent in developmental transcription factors, a domain found in the promyelocytic leukemia-associated SP100 proteins, and a zinc finger homology domain associated with the AML1/MTG8 oncoprotein. Although NUDR mRNA displayed a wide tissue distribution in rats, elevated levels of protein were only observed in testicular germ cells, developing fetus, and transformed cell lines. Nuclear localization of NUDR was demonstrated by immunocytochemistry and by a green fluorescent protein-NUDR fusion protein. Site-directed mutagenesis of a nuclear localization signal resulted in cytoplasmic localization of the protein and eliminated NUDR-dependent transcriptional activation. Recombinant NUDR protein showed affinity for the RARE in mobility shifts; however it was efficiently displaced by retinoic acid receptor (RAR)/retinoid X receptor (RXR) complexes. In transient transfections, NUDR produced up to 26-fold inductions of a human proenkephalin promoter-reporter plasmid, with minimal effects on the promoters for prodynorphin or thymidine kinase. Placement of a RARE on the proenkephalin promoter increased NUDR-dependent activation to 41-fold, but this RARE-dependent increase was not transferable to a thymidine kinase promoter. Recombinant NUDR protein showed minimal binding affinity for proenkephalin promoter sequences, but was able to select DNA sequences from a random oligonucleotide library that had similar core-binding motifs (TTCG) as those recognized by DEAF-1. This motif is also present between the half-sites of several endogenous RAREs. The derived consensus-binding motif recognized by NUDR (TTCGGGNNTTCCGG) was confirmed by mobility shift and deoxyribonuclease I (DNase I) protection assays; however, the consensus sequence was also unable to confer NUDR-dependent transcriptional activation to the thymidine kinase promoter. Our data suggests that NUDR may activate transcription independently of promoter binding, perhaps through protein-protein interaction with basal transcription factors, or by activation of secondary factors. The sequence and functional similarities between NUDR and DEAF-1 suggest that NUDR may also act as a cofactor to regulate the transcription of genes during fetal development or differentiation of testicular cells.

L5 ANSWER 14 OF 21 MEDLINE DUPLICATE 7
AN 1999084763 MEDLINE
DN 99084763 PubMed ID: 9869418
TI Alfin1, a novel zinc-finger protein in alfalfa roots that binds to promoter elements in the salt-inducible MsPRP2 gene.
AU Bastola D R; Pethe V V; Winicov I
CS Department of Microbiology, School of Medicine, University of Nevada Reno, 89557, USA.
SO PLANT MOLECULAR BIOLOGY, (1998 Dec) 38 (6) 1123-35.
Journal code: 9106343. ISSN: 0167-4412.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF028841
EM 199901
ED Entered STN: 19990202
Last Updated on STN: 19990202
Entered Medline: 19990115
AB Alfin1 cDNA, obtained by differential screening of a poly(A)+ library from

salt-tolerant alfalfa **cells**, encodes a novel protein with a Cys4 and His/Cys3 putative zinc-binding domain that suggests a possible role for this protein in transcriptional regulation. We have expressed the cDNA in *Escherichia coli* and show that the recombinant Alfin1 protein binds DNA in a sequence-specific manner. The DNA recognition sequence was determined from individual clones isolated after four rounds of **random** oligonucleotide selection in gel retardation **assays**, coupled with PCR amplification of the selected sequences. The consensus binding site for Alfin1 is shown to contain two to five G-rich triplets with the conserved core of GNGGTG or GTGGNG in clones showing high-efficiency binding. DNA binding of the recombinant Alfin1 was inhibited by EDTA. Alfin1 mRNA was found predominantly in alfalfa roots. Growth of salt-sensitive *Medicago sativa* L on 171 mM NaCl led to a slight decrease in Alfin1 mRNA, while the salt-tolerant plants showed no decrease in Alfin1 mRNA levels. Interestingly, recombinant Alfin1 binds efficiently to three fragments of the MsPRP2 promoter, each containing consensus sequences identified by the **random** oligonucleotide selection. Since MsPRP2 transcripts were shown to be root-specific and accumulated in alfalfa roots in a salt-inducible manner, Alfin1 may play a role in the regulated expression of MsPRP2 in alfalfa roots and contribute to salt tolerance in these plants.

L5 ANSWER 15 OF 21 MEDLINE
 AN 1998108016 MEDLINE
 DN 98108016 PubMed ID: 9443972
 TI Identification of the DNA sequence that interacts with the gut-enriched Kruppel-like factor.
 AU Shields J M; Yang V W
 CS Department of Medicine and Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.
 SO NUCLEIC ACIDS RESEARCH, (1998 Feb 1) 26 (3) 796-802.
 Journal code: 0411011. ISSN: 0305-1048.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 OS GENBANK-AF117109; GENBANK-U20344
 EM 199803
 ED Entered STN: 19980319
 Last Updated on STN: 20000303
 Entered Medline: 19980312
 AB The gut-enriched Kruppel-like factor (GKLF) is a recently identified eukaryotic transcription factor that contains three C2H2zinc fingers. The amino acid sequence of the **zinc finger** portion of GKLF is closely related to several Kruppel proteins, including the lung Kruppel-like factor (LKLF), the erythroid Kruppel-like factor (EKLF) and the basic transcription element binding protein 2 (BTEB2). The DNA sequence to which GKLF binds has not been definitively established. In the present study we determined the DNA binding sequence of GKLF using highly purified recombinant GKLF in a target detection **assay** of an oligonucleotide library consisting of **random** sequences. Upon repeated rounds of selection and subsequent characterization of the selected sequences by base-specific mutagenesis a DNA with the sequence 5'-G/AG/AGGC/TGC/T-3' was found to contain the minimal essential binding site for GKLF. This sequence is present in the promoters of two previously characterized genes: the CACCC element of the beta-globin gene, which interacts with EKLF, and the basic transcription element (BTE) of the CYP1A1 gene, which interacts with Sp1 and several Sp1-like transcription factors. Moreover, the selected GKLF binding sequence was capable of mediating transactivation of a linked reporter gene by GKLF in co-transfection experiments. Our results establish GKLF as a sequence-specific transcription factor likely involved in regulation of expression of endogenous genes.

AN 96315626 MEDLINE
 DN 96315626 PubMed ID: 8754800
 TI Gfi-1 encodes a nuclear **zinc finger** protein that binds DNA and functions as a transcriptional repressor.
 AU Zweidler-Mckay P A; Grimes H L; Flubacher M M; Tsichlis P N
 CS Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.
 NC CA06927 (NCI)
 CA56110 (NCI)
 CA59302 (NCI)
 SO MOLECULAR AND CELLULAR BIOLOGY, (1996 Aug) 16 (8) 4024-34.
 Journal code: 8109087. ISSN: 0270-7306.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199609
 ED Entered STN: 19961008
 Last Updated on STN: 19970203
 Entered Medline: 19960920
 AB The Gfi-1 proto-oncogene encodes a **zinc finger** protein with six C2H2-type, C-terminal **zinc finger** motifs and is activated by provirus integration in T-cell lymphoma lines selected for interleukin-2 independence in culture and in primary retrovirus-induced thymomas. Gfi-1 expression in adult animals is restricted to the thymus, spleen, and testis and is enhanced in mitogen-stimulated splenocytes. In this report, we show that Gfi-1 is a 55-kDa nuclear protein that binds DNA in a sequence-specific manner. The Gfi-1 binding site, TAAATCAC(A/T)GCA, was defined via **random** oligonucleotide selection utilizing a bacterially expressed glutathione S-transferase-Gfi-1 fusion protein. Binding to this site was confirmed by electrophoretic mobility shift **assays** and DNase I footprinting. Methylation interference analysis and electrophoretic mobility shift **assays** with mutant oligonucleotides defined the relative importance of specific bases at the consensus binding site. Deletion of individual **zinc fingers** demonstrated that only **zinc fingers** 3, 4, and 5 are required for sequence-specific DNA binding. Potential Gfi-1 binding sites were detected in a large number of eukaryotic promoter-enhancers, including the enhancers of several proto-oncogenes and cytokine genes and the enhancer of the human cytomegalovirus (HCMV) major immediate-early promoter, which contains two such sites. HCMV major immediate-early-chloramphenicol acetyltransferase reporter constructs, transfected into NIH 3T3 fibroblasts, were repressed by Gfi-1, and the repression was abrogated by mutation of critical residues in the two Gfi-1 binding sites. These results suggest that Gfi-1 may play a role in HCMV biology and may contribute to oncogenesis and T-cell activation by repressing the expression of genes that inhibit these processes.

L5 ANSWER 17 OF 21 MEDLINE
 AN 96425271 MEDLINE
 DN 96425271 PubMed ID: 8827714
 TI The MDM2 oncoprotein binds specifically to RNA through its RING finger domain.
 AU Elenbaas B; Dobbelstein M; Roth J; Shenk T; Levine A J
 CS Department of Molecular Biology, Princeton University, NJ 08544-1014, USA.
 SO MOLECULAR MEDICINE, (1996 Jul) 2 (4) 439-51.
 Journal code: 9501023. ISSN: 1076-1551.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199701
 ED Entered STN: 19970128
 Last Updated on STN: 19970128
 Entered Medline: 19970102

AB BACKGROUND: The cellular mdm2 gene has transforming activity when overexpressed and is amplified in a variety of human tumors. At least part of the transforming ability of the MDM2 protein is due to binding and inactivating the p53 tumor suppressor protein. Additionally, this protein forms a complex in vivo with the L5 ribosomal protein and its associated 5S ribosomal RNA and may be part of a ribosomal complex. MATERIALS AND METHODS: A RNA homopolymer binding **assay** and a SELEX procedure have been used to characterize the RNA-binding activity of MDM2. RESULTS: The MDM2 protein binds efficiently to the homopolyribonucleotide poly(G) but not to other homopolyribonucleotides. This binding is independent of the interaction of MDM2 with the L5 protein, which occurs through the central acidic domain of MDM2. An RNA SELEX procedure was performed to identify specific RNA ligands that bind with high affinity to the human MDM2 (HDM2) protein. After 10 rounds of selection and amplification, a subset of RNA molecules that bound efficiently to HDM2 was isolated from a **randomized** pool. Sequencing of these selected ligands revealed that a small number of sequence motifs were selected. The specific RNA binding occurs through the RING finger domain of the protein. Furthermore, a single amino acid substitution in the RING finger domain, G446S, completely abolishes the specific RNA binding. CONCLUSIONS: These observations, showing that MDM2 binds the L5/5S ribosomal ribonucleoprotein particle and can also bind to specific RNA sequences or structures, suggest a role for MDM2 in translational regulation in a **cell**.

L5 ANSWER 18 OF 21 MEDLINE DUPLICATE 9

AN 95198747 MEDLINE

DN 95198747 PubMed ID: 7891721

TI DNA-binding specificity of NGFI-A and related **zinc finger** transcription factors.

AU Swirnoff A H; Milbrandt J

CS Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110.

NC P01 CA53514 (NCI)

SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Apr) 15 (4) 2275-87.
Journal code: 8109087. ISSN: 0270-7306.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199504

ED Entered STN: 19950427

Last Updated on STN: 19950427

Entered Medline: 19950420

AB NGFI-A is the prototypic member of a family of immediate-early gene-encoded transcription factors which includes NGFI-C, Egr3, and Krox20. These proteins possess highly homologous DNA-binding domains, composed of three Cys2-His2 **zinc fingers**, and all bind to and activate transcription from the sequence GCGGGGGCG. We used a PCR-mediated **random** site selection protocol to determine whether other sites could be bound by these proteins and the extent to which their binding site preferences are similar or different. The high-affinity consensus sites generated from the selection data are similar, and the combined consensus sequence is T-G-C-G-T/g-G/A-G-G-C/a/t-G-G/T (lowercase letters indicate bases selected less frequently). Using gel shift **assays**, we found that sequences that diverge from the consensus were bound by NGFI-A, confirming that there is greater variability in binding sites than has generally been acknowledged. We also provide evidence that protein-DNA interactions not noted, or whose importance was not apparent from the X-ray cocrystal structure of the NGFI-A **zinc fingers** complexed with DNA, contribute significantly to the binding energy of these proteins and confirm that an optimal site is at least 10 instead of 9 nucleotides in length. In contrast to the similarities in binding specificity among these proteins we found that while NGFI-A, Egr3, and Krox20 have comparable DNA binding affinities and

kinetics of dissociation, the affinity of NGFI-C is more than threefold lower. This could result in differential regulation of target genes in **cells** where NGFI-C and the other proteins are coexpressed. Furthermore, we show that this affinity difference is a property not of the **zinc fingers** themselves but rather of the protein context of the DNA-binding domain.

L5 ANSWER 19 OF 21 MEDLINE DUPLICATE 10
 AN 95032121 MEDLINE
 DN 95032121 PubMed ID: 7945383
 TI Recognition DNA sequence of a novel putative transcription factor, BCL6.
 AU Kawamata N; Miki T; Ohashi K; Suzuki K; Fukuda T; Hirose S; Aoki N
 CS First Department of Internal Medicine, Tokyo Medical and Dental University, Japan.
 SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1994 Oct 14) 204 (1) 366-74.
 Journal code: 0372516. ISSN: 0006-291X.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199411
 ED Entered STN: 19941222
 Last Updated on STN: 19980206
 Entered Medline: 19941123
 AB The BCL6 gene involved in the 3q27 translocation associated with B-cell lymphomas encodes a novel Cys2-His2 **zinc finger** protein. We generated a fusion protein of glutathione S-transferase and **zinc finger** domain of BCL6 to determine recognition sequences of BCL6 with polymerase chain reaction using **random** oligonucleotides of 26 bases as a ligand. A consensus of 14 nucleotides consisting of (T/A)NCTTTCNAGG(A/G)AT was identified in the recognition sequences. In a gel mobility shift **assay**, the probe containing the 14-nucleotide recognition sequence formed a complex with the fusion protein and nuclear proteins from Burkitt's **cell** lines overexpressing the BCL6 transcripts. The consensus sequence was protected from the digestion by nuclease in a DNase I footprinting **assay**. In conclusion, BCL6 may be involved in tumorigenesis by binding to the consensus sequences of the other genes.

L5 ANSWER 20 OF 21 MEDLINE
 AN 93309433 MEDLINE
 DN 93309433 PubMed ID: 8321207
 TI DNA-binding specificity of GATA family transcription factors.
 AU Merika M; Orkin S H
 CS Division of Hematology/Oncology, Children's Hospital, Dana-Farber Cancer Institute, Boston, Massachusetts.
 SO MOLECULAR AND CELLULAR BIOLOGY, (1993 Jul) 13 (7) 3999-4010.
 Journal code: 8109087. ISSN: 0270-7306.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199307
 ED Entered STN: 19930813
 Last Updated on STN: 19930813
 Entered Medline: 19930730
 AB GATA-binding proteins constitute a family of transcription factors that recognize a target site conforming to the consensus WGATAR (W = A or T and R = A or G). Here we have used the method of polymerase chain reaction-mediated **random** site selection to assess in an unbiased manner the DNA-binding specificity of GATA proteins. Contrary to our expectations, we show that GATA proteins bind a variety of motifs that deviate from the previously assigned consensus. Many of the nonconsensus sequences bind protein with high affinity, equivalent to that of

conventional GATA motifs. By using the selected sequences as probes in the electrophoretic mobility shift **assay**, we demonstrate overlapping, but distinct, sequence preferences for GATA family members, specified by their respective DNA-binding domains. Furthermore, we provide additional evidence for interaction of amino and carboxy fingers of GATA-1 in defining its binding site. By performing cotransfection experiments, we also show that transactivation parallels DNA binding. A chimeric protein containing the finger domain of *areaA* and the activation domains of GATA-1 is capable of activating transcription in mammalian **cells** through GATA motifs. Our findings suggest a mechanism by which GATA proteins might selectively regulate gene expression in **cells** in which they are coexpressed.

L5 ANSWER 21 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 AN 1990:517369 BIOSIS
 DN BA90:134645
 TI ROLE OF THE ZINC-II IONS IN THE STRUCTURE OF THE THREE-FINGER DNA BINDING DOMAIN OF THE SP1 TRANSCRIPTION FACTOR.
 AU KUWAHARA J; COLEMAN J E
 CS DEP. MOLECULAR BIOPHYSICS BIOCHEM., YALE UNIV., NEW HAVEN, CONN. 06510.
 SO BIOCHEMISTRY, (1990) 29 (37), 8627-8631.
 CODEN: BICHAW. ISSN: 0006-2960.
 FS BA; OLD
 LA English
 AB The transcription factor Sp1 from Hela **cells** contains near the C-terminus of this protein of 778 amino acids three contiguous repeats of an amino acid sequence, -Cys-X4-Cys-X12-His-X3-His-, typical of the Cys2-His2-type **zinc-finger** DNA binding domain first found in transcription factor TFIIA. A DNA sequence corresponding to the codons from residue 614 to residue 779 of Sp1 (encompassing the three **zinc-finger** motifs) has been cloned and overproduced in *Escherichia coli*. The fragment of Sp1 containing the C-terminal 165 residues plus 2 from the cloning vector, designated Sp1 (167*), can be extracted with 5 M urea and then refolded in the presence of Zn(II) to a protein of specific conformation containing 3.0 +/- 0.2 mol of tightly bound Zn(II)/mol of protein. Gel retardation **assays** using a labeled 14-bp DNA sequence containing a consensus Sp1 binding site show that the refolded Zn(ii) protein specifically recognizes the "GC box" sequence in the presence of a large excess of calf thymus DNA. Treatment of Zn(II)Sp1(167*) with 10 mM EDTA results in removal of Zn(II) and the formation of an apoprotein which does not specifically recognize DNA. Cd(II) can be exchanged for Zn(II) in the refolded protein with full retention of specific DNA recognition. This is the first Cys2His2-type "finger" protein where this substitution has been accomplished. Titration of the Zn(II) protein with 6 mol of p-mercuribenzenesulfonate/mol of protein results in the complete release of the three Zn(II) ions. Results of Zn(II) is highly cooperative. Reaction of only two of the sulfhydryl zinc ligands with the organic mercurial releases 75% of the Zn(II), suggesting that the Zn(II)-induced folding of the three fingers is probably cooperative. Circular dichroism shows the Zn(II)3 protein to contain .apprx.20% .alpha.-helix, .apprx.-20% .beta.-sheet, and .apprx.60% **random** coil as the secondary structure of the **zinc-finger** domain. A large part of the second structure is lost when the metal ions are removed.

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L1 13039 S ZINC FINGER
L2 214 S L1 AND RANDOM?
L3 108 S L2 AND (CELL OR VIVO)
L4 31 S L2 AND (SCREEN OR ASSAY) AND CELL
L5 21 DUPLICATE REMOVE L4 (10 DUPLICATES REMOVED)

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1.21	17.94

FULL ESTIMATED COST

STN INTERNATIONAL LOGOFF AT 15:30:00 ON 24 JUL 2002